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Solubility of drugs in the presence of gelatin: effect of drug lipophilicity and degree of ionization

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Abstract

The solubility of seven drugs (nitrofurantoin, chlorothiazide, phenobarbital, prednisolone, griseofulvin, diazepam and piroxicam) in the absence and presence of gelatin was measured, at three different pH values (3.7, 5.0 and 7.0) at 37°C. Drugs studied had different physicochemical properties (log P, pK_a, aqueous solubility) and their solubility in presence of 0.1 and 0.5% (w/v) hydrolyzed (and in some cases common) gelatin was estimated. Results show that the solubility of all drugs is significantly enhanced, especially in the presence of 0.5% gelatin. This gelatin-induced enhancement in drug solubility is higher in the pH in which acidic drugs are less ionized, especially for the less lipophilic acidic drugs (nitrofurantoin, chlorothiazide). In all cases, drug solubility in presence of gelatin is correlated with their aqueous solubility. Therefore, the established relationships between aqueous and gelatin solubility can be employed to derive an estimate of the drug solubility in presence of gelatin once its aqueous solubility is known. With the exception of piroxicam which is highly ionized and phenobarbital which is relatively soluble, there seems to be a tendency for larger gelatin-induced increases in solubility as drug lipophilicity increases or aqueous solubility decreases.

Keywords: Solubility; Gelatin; Hydrolyzed gelatin; Drugs

1. Introduction

Solubility of drugs in aqueous media is a key factor highly influencing their dissolution rate and bioavailability following oral administration. The effect of several substances — physiologically present in the gastrointestinal tract (bile salts, etc.) or not (food ingredients or drug excipients) — on drug solubility has been the subject of several research projects in the past.

Gelatin, a heterogeneous mixture of water-soluble proteins of high average molecular weight derived from collagen, has many applications in food and pharmaceutical industry. In addition to conventional drug formulations, recently gelatin has been used in modern systems designed for controlled release of drugs (Kim and Fassihi, 1997; Cortesi et al., 1999; Huang et al., 1999; Kantaria et al., 1999).

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In early studies it was found that the bioavailability of digoxin when administered as a soft gelatin capsule was about 13–20% higher of that obtained after administration of tablets and, even more surprisingly, higher than the bioavailability of a digoxin solution (Johnson et al., 1976). Since digoxin dissolution is the rate limiting step for its absorption, it was suggested that perhaps the soft gelatin wall influences digoxin solubility in a positive way (Johnson et al., 1986). In other studies (Imai et al., 1989, 1990; Kimura et al., 1990), it was observed that enzymatically hydrolyzed gelatin, which is highly water-soluble and has mild surface activity, improved the dissolution and/or absorption rate and bioavailability of several poorly water-soluble drugs.

In general, the interaction between drugs and gelatin has not been studied in extent, particularly in view of the possibility that gelatin may have an effect on the gastrointestinal absorption of sparingly soluble drugs, with the exception of a study conducted earlier in which the binding and other physicochemical interactions between gelatin and four acidic azo dyes were investigated (Gautam and Schott, 1994a,b). However, in these latter studies the purpose was to investigate the effect that acidic drugs have on gelatin properties and to what extent drugs may influence gelatin properties and thereby their release from gelatin (soft or hard) capsules or microcapsules. Therefore, the focus was on gelatin properties in the presence of drugs and not vice-versa.

The objective of the present study is to investigate the possible effect of gelatin on the solubility of drugs, at various pH values. Since gelatin will be hydrolyzed anyway in the gastrointestinal tract, hydrolyzed gelatin which is also easier to work with (substantially higher solubility and lower surface activity) was used. Seven drugs with differences in their basic physicochemical properties ($\log P$, pK_a, and aqueous solubility) were chosen, and their solubility was measured at three pH values (in the range of the pH values of the various parts of the GI-tract) 3.7, 5.0 and 7.0, in the absence and presence of two gelatin concentrations (0.1 and 0.5% w/v).

2. Materials and methods

Diazepam (DZP) was a kind gift from Adelco, Athens, Greece. Griseofulvin (GFV), Prednisolone (PR), Nitrofurantoin (NTF), Chlorothiazide (CT), Piroxicam (PX) and Phenobarbital (PNB) were purchased by Sigma (Saint Louis, MO). All drugs were found to be $>99\%$ pure, after injecting in an HPLC column. Common gelatin and gelatin hydrolysate were purchased from Sigma (Saint Louis, MO). All other reagents used were of analytical grade and were purchased from Merck (Germany).

A Shimadzu UV-1205 UV–VIS spectrophotometer was utilized for determination of drugs in their solutions in presence and absence of gelatin. A Julabo shaking and thermostated water incubator was used throughout the study.

2.1. Preparation of buffers

Three different buffers were used, in all cases, with high concentrations in order to diminish the possibility that gelatin may influence the final pH of the gelatin solutions prepared. Indeed, the final pH of all gelatin solutions used was measured and found to be unchanged.

Each buffer was prepared as follows:

Acetate buffer-pH 3.7: 200 ml of a 0.2 M solution of sodium acetate were mixed with 1800 ml of 0.2 M acetic acid.

Acetate buffer-pH 5.0: 1400 ml of a 0.2 M solution of sodium acetate were mixed with 600 ml of 0.2 M acetic acid.

Phosphate buffer-pH 7.0: 1220 ml of a 0.2 M solution of dibasic sodium phosphate were mixed with 780 ml of a 0.2 M solution of monobasic sodium phosphate.

2.2. Drug calibration curves

In order to determine the solubility of drugs, standard curves were prepared for each drug in each medium and in each gelatin concentration (in the final solution). The concentration range of the standard solutions prepared was always between 2 and 20 ppm.

Absorbance values of standard solutions and samples were measured at $\lambda = 375$ nm for NTF, 270 nm for CT, 254 nm for PNB, 248 nm for PR, 280 nm for GFV, 357 for PX and 230 nm for DZP, and drug concentration in the samples was calculated after preparing the appropriate calibration curve for each drug.

In all cases, standard curves prepared for the determination of drug concentration in samples, were linear with $R > 0.997$, while there was no significant difference between the slope's of the curves prepared in plain buffer and gelatin containing buffers. The blank gelatin sample, prepared in the buffer used in each case, did not have any significant absorbance (even the highest concentration present in the samples (0.1% w/v)), with the exception of DZP, for which the blank value was measured and subtracted from the absorbance value of each sample and standard solution.

2.3. Determination of drug solubility

The solubility of each drug investigated was determined in three different pH values (3.7, 5.0, 7.0), representative of various sections of the gastrointestinal tract), in absence (aqueous solubility) and in presence of gelatin. Two different concentrations, 0.1 and 0.5% (w/v) of soluble gelatin (common gelatin was also used in some cases) were used. Soluble or low-molecular weight gelatin (average molecular weight, 6000) is a hydrolysate of gelatin with mild surface activity and high aqueous solubility (aqueous solubility differs by more than 20% from that of usual gelatin).

For determination of drug solubility drug (powder) was added in excess in conical flasks which contained 10 ml of the medium studied in each case. Each solubility value was determined in triplicate, at least. The flasks were placed in a thermostated water bath at 37.0 ± 0.5°C agitated at 60 rpm, for at least 24 h. After this period, 1 ml was withdrawn, filtered, diluted five times with the appropriate buffer and drug concentration was determined spectrophotometrically.

2.4. Calculation of corrected log P values

In order to correlate drug lipophilicity with drug solubility in the presence of gelatin, drug log P values were corrected for ionization at different pH values by the equation:

$$\log P = \log D + \text{correction term}$$

where log D values were taken from Hanch and Leo, 1979, and the correction term is calculated as follows:

$\log(1 + 10^{\mathrm{pH} - \mathrm{p}Ka})$, for mono-acids.

$\log(1 + 10^{\mathrm{p}Ka - \mathrm{pH}})$, for mono-bases.

$\log(1 + 10_1^{\mathrm{pH} - \mathrm{p}Ka} + 10^{2\mathrm{pH} - \mathrm{pK_a}1 - \mathrm{pK_a}}2)$, $\mathrm{p}Ka_1 < \mathrm{p}Ka_2$, for di-acids.

$\log(1 + 10^{\mathrm{p}Ka}1^{-\mathrm{pH}} + 10^{\mathrm{p}Ka}1^{+\mathrm{p}Ka}2^{-2\mathrm{pH}})$, $\mathrm{p}Ka_1 > \mathrm{p}Ka_2$, for di-bases.

The physicochemical properties (log P corrected values and % ionized drug at the various pH studied) of all the drugs studied herein, are presented in Table 1.

3. Results and discussion

Despite the numerous pharmaceutical and food related uses of gelatin, scant attention has been devoted to its interaction with drugs, especially when the effect of gelatin on bioavailability-related drug properties is considered. Kimura et al. (1990), have shown that low molecular weight hydrolyzed gelatin increases the dissolution rate of acidic, basic and neutral drugs in a kneaded mixture, while Imai et al. (1989), confirmed the enhancement of the dissolution rates of poorly water-soluble drugs by water-soluble gelatin. Although in most of these latter studies the solubility of the drugs used in presence of gelatin was not systematically examined, the enhanced dissolution was attributed to increase of drug wettability by gelatin. Herein, we investigated the effect of soluble gelatin on drug solubility.

Drug solubility in absence and presence of gelatin as a function of pH is shown in Fig. 1. In most cases the solubility of the drugs is significantly increased (20–130%) when compared with their aqueous solubility, even in the presence of the low gelatin concentration (0.1%). As antici-

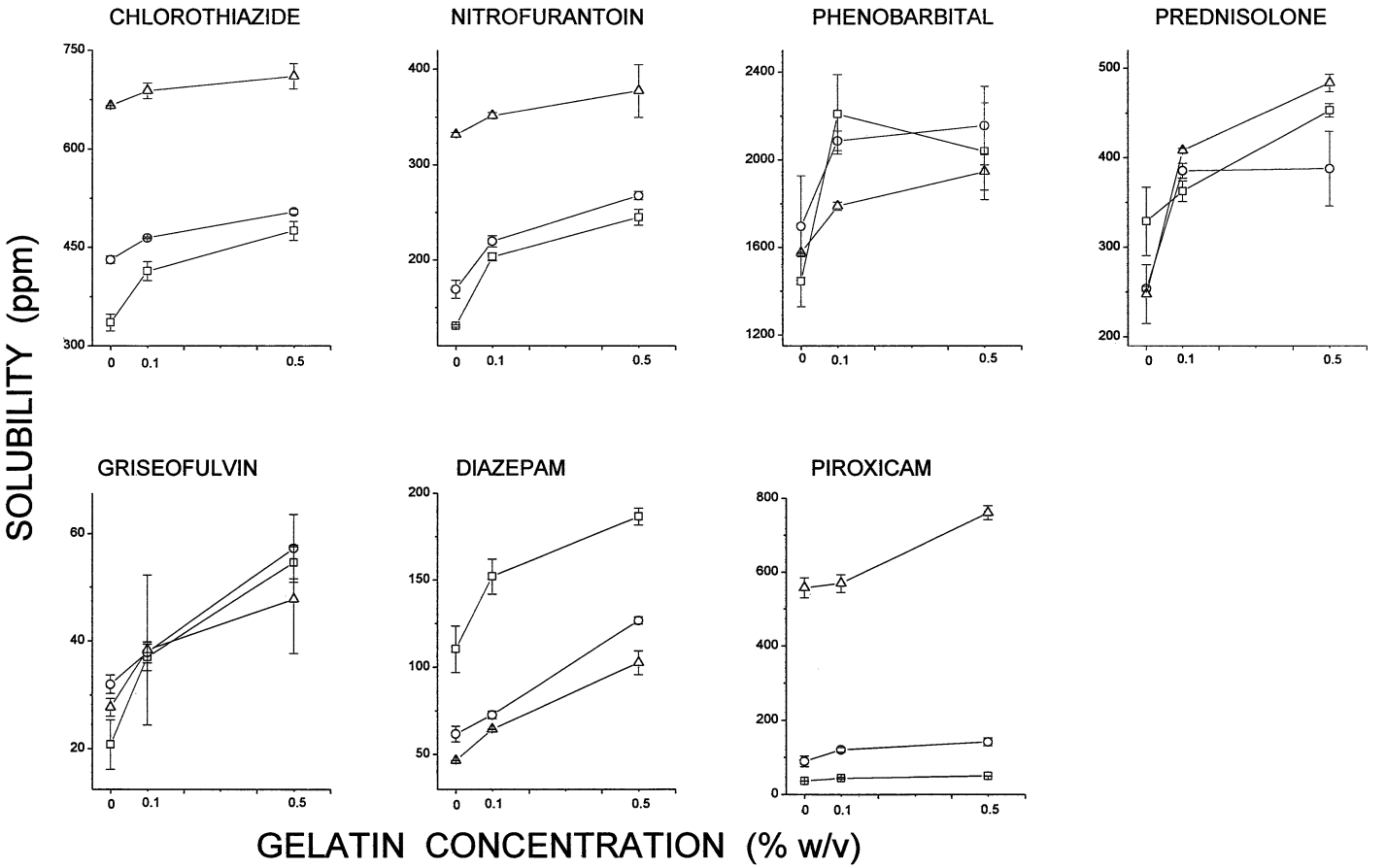

Fig. 1. Plots of aqueous solubility of drugs at various pH values at 37°C, as a function of soluble gelatin concentration. Key: squares, pH 3.7; circles, pH 5.0 and triangles, pH 7.0.

Table 1
Physicochemical properties of the drugs studied, as a function of pH

Drug	pK_a	% Ionized drug			log P[a]		
		pH			pH		
		3.7	5.0	7.0	3.7	5.0	7.0
NTF	7.2	0.1	0.6	38.7	−0.47	−0.47	−0.68
CT	6.7–9.5	0.1	1.9	66.7	−0.24	−0.25	−0.72
PNB	7.4–11.8	0.01	0.49	28.47	1.47	1.47	1.33
PR	–	0	0	0	1.62	1.62	1.62
GFV	>9	0.01	0.01	1	2.18	2.18	2.18
DZP	3.4	33.38	2.45	0.025	2.81	2.98	2.99
PX	6.3	0.3	4.8	83.4	3.06	3.04	2.28

[a] log P: corrected values were calculated as presented in Section 2.

the low gelatin concentration (0.1%). As anticipated, the pH value of the solution used has a profound effect on the solubility values of the drugs which are highly ionized in the pH range studied, i.e. Nitrofurantoin, Chlorothiazide, Piroxicam and Diazepam, a moderate effect on the solubility of Phenobarbital, which is ionized at pH 7.0 ($\sim$25%), and, more or less, no effect on the solubility of non-ionized Griseofulvin and Prednisolone. Nevertheless, if carefully observing the results presented in Fig. 1, it is evident that the gelatin-induced increase in solubility of the acidic drugs studied is higher when the drugs are not ionized (especially clear in the cases of NTF and CT for which increases in drug solubility are profoundly higher at pH 3.7 and 5.0 ($\sim$10–80%) in which they are non-ionized, than at pH 7.0 ($\sim$0–13%) in which they are both considerably ionized (Table 1)). In addition, it seems that drugs with lower aqueous solubility are affected more by the presence of gelatin, a fact that correlates well with the above mentioned role of ionization (since drug solubility is higher when the drug is ionized). Indeed, increases in solubility as high as $\sim$120 and 135% for Diazepam (pH 7.0) and Griseofulvin (pH 3.7), respectively are observed in presence of 0.5% gelatin.

In order to have a better picture of the effect of physicochemical properties of drugs on the way by which gelatin affects their solubility, the percent increase of solubility in presence of the high gelatin concentration was calculated in all cases (compared with the aqueous solubility in the corresponding pH value). As presented in Fig. 2, when these values are plotted against the corrected log P values calculated for each drug at each pH value studied (Table 1), it seems that the effect of gelatin is higher for the more lipophilic drugs. This is particularly true if two of the drugs are not considered, i.e. Phenobarbital, which is anyway comparably soluble, and Piroxicam, which is highly ionized.

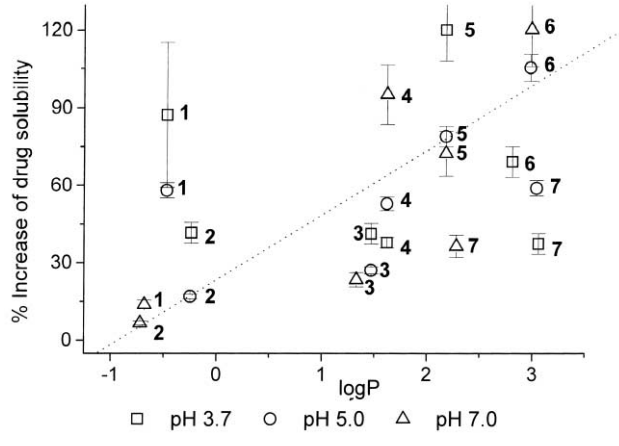

Fig. 2. Plot of the % increase in solubility in the presence of 0.5% (w/v) soluble gelatin as a function of log P of each drug. The log P value of each drug was corrected for ionization as described under Section 2 (corrected values are listed in Table 1). Each drug is presented by the number assigned to it in Table 1, i.e. 1 = NTF, 2 = CT, 3 = PNB, 4 = PR, 5 = GFV, 6 = DZP, 7 = PX. Key: squares, pH 3.7; circles, pH 5.0 and triangles, pH 7.0.

Table 2
Established relationships between aqueous solubility of drugs and solubility in presence of 0.1 and 0.5% soluble gelatin, at pH 3.7, 5.0 and 7.0[a]

Case (pH–gelatin concentration)	*A*	*B*	*R*
pH 3.7–0.1%	–	1.535	0.9917
pH 3.7–0.5%	21.259	1.394	0.9994
pH 5.0–0.1%	6.56	1.223	0.9984
pH 5.0–0.5%	33.74	1.246	0.9989
pH 7.0–0.1%	13.27	1.105	0.9943
pH 7.0–0.5%	46.518	1.188	0.9908

[a] In all cases the relationship is linear: Solubility in gelatin $= A + B \times$ (aqueous solubility). The values of A and B, as well as the correlation coefficient in each case are given above.

As mentioned above, in some cases drug solubility was also determined in presence of common gelatin. In general, the values measured were slightly lower (not shown), while, in this case, drug ionization did not seem to play an important role on the gelatin-induced increase of solubility for the drugs with acidic properties.

When the aqueous solubility is plotted against solubility in gelatin solutions, a linear correlation with high correlation coefficients (R ranges from 0.9908–09994) between the two sets of values exists, in all cases (Table 2). This is easily observed from Fig. 3 in which the values measured at pH 5.0 are plotted, while similar plots are obtained with solubility values determined in pH 3.7 and 7.0 (not shown). However, a closer observation of these plots may reveal that if the point for Phenobarbital, which is comparably highly soluble, is omitted, the remaining points form two groups of data (insert in top graph of Fig. 3). The first group consists of sparingly soluble drugs (with aqueous solubility < 100 ppm) for which

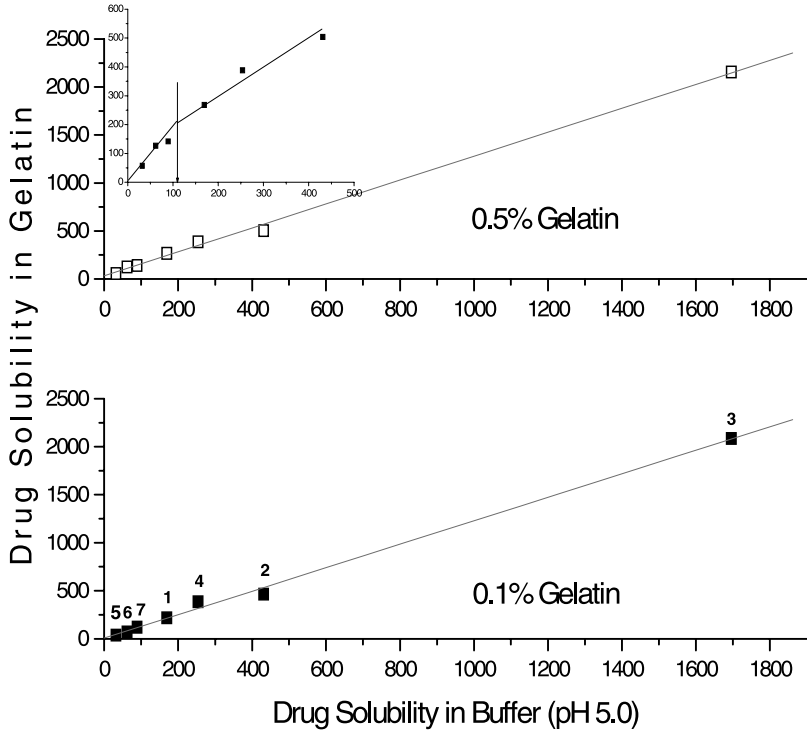

Fig. 3. Typical plots of the relationship between aqueous solubility and solubility in presence of soluble gelatin at 37°C, at pH 5.0. In the lower figure, each drug is presented by the number assigned to it in Table 1, i.e. 1 = NTF, 2 = CT, 3 = PNB, 4 = PR, 5 = GFV, 6 = DZP, 7 = PX. Lower figure: 0.1% (w/v) gelatin; upper figure: 0.5% (w/v) gelatin. Insert: magnification of part of the upper figure.

the relative increase in gelatin solubility is higher. The second group of drugs consists of drugs for which solubility in gelatin solutions levels off. The aqueous solubility which separates the two groups of drugs is >100 ppm for the values measured at pH 5.0, while <100 ppm and ~ 200 ppm for those at pH 3.7 and 7.0, respectively. It is interesting that a similar behavior has been demonstrated before for the relationship between aqueous and milk solubility of drugs, when drugs of similar aqueous solubility were studied (Macheras et al., 1990). In fact, in good agreement with the present results, also these aqueous/milk solubility relationships could not extend to drugs with aqueous solubilities higher than 500 ppm (Macheras et al., 1989, 1990). However, this is not enough proof to indicate that perhaps there are other similarities between the way gelatin and milk components enhance the solubility of such sparingly soluble drugs. In addition, the increases in drug solubility by gelatin (although a low amount of gelatin was used), are very low compared with the milk-induced increases measured earlier (Macheras et al., 1989, 1990). Nevertheless, if the linear relationships established here (Table 2) are used for prediction of solubility of drugs in presence of gelatin, the values obtained will be very close to those measured, in all cases (different gelatin concentration or pH). Therefore, these relationships between aqueous solubility of drugs and their solubility in presence of gelatin (0.1 or 0.5% w/v) can be employed to derive an estimate of the drug solubility in presence of gelatin once its aqueous solubility is known.

Summarizing, the results of this study (Figs. 1 and 2) reveal that the solubility of all the drugs studied in gelatin solutions is increased when compared with their corresponding (at the specific pH value) aqueous solubility. The percent increase in drug solubility as a result of the presence of a small amount of gelatin is in general higher for the sparingly soluble and/or highly lipophilic drugs. Therefore, although no explanations may be provided at this point about the reason for gelatin enhanced drug solubility, the possibility that enhanced drug absorption and bioavailability observed after administration of gelatin-containing formulations, may be at least partially attributed to enhanced drug solubility due to gelatin–drug interactions, should not be undermined. Furthermore, it is interesting to continue studies, especially with more lipophilic drugs.

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