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Solubility of the antibiotic Penicillin G in supercritical carbon dioxide

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Abstract

The solubility of the antibiotic Penicillin G in supercritical carbon dioxide was measured at pressures from 100 to 350 bar and temperatures from 313.15 to 333.15 K using a dynamic flow apparatus. Physical properties and critical parameters of Penicillin G were estimated using Fedor's and Ambrose's group contribution methods. The experimental data were correlated using the Redlich–Kwong and Soave–Redlich–Kwong equations of state, with Lorent–Berthelot mixing rules. A second order empirical model to correlate solubility directly with temperature and pressure was used.

Keywords: Antibiotic; Equation of state; Penicillin G; Solubility; Supercritical fluid

1. Introduction

β-lactams antibiotics represent the most important group of commercially available antibiotics. These compounds are divided into two chemical types: penicillins and cephalosporins. The penicillins are high molecular weight polyfunctional substances which are characterized by their high polarity, low volatility, and thermal liability [1]. The penicillins differ from one another in the nature of the amide side chain. Penicillin G is the most used currently as an antimicrobial agent.

The common nucleus of the penicillin molecules is the 6-aminopenicilanic acid (6-APA) [2]. The production of 6-APA in quantity is carried out to remove the lateral chain of benzylpenicillin (Penicillin G), opening the way to the semisynthesis of a series of penicillins that cannot be obtained by fermentation; thus, it is important in obtaining penicillins [3].

The classical production of these antibiotics comprises a series of separation and purification processes, with a series of organic solvent extraction and precipitation processes, to conclude with product crystallization. The solute–solvent separation stage is very important due to the large volume of organic solvent to be eliminated, and the difficulties in the solvent separation and recovery stage [4].

The interest in the supercritical fluid extraction (SFE) lies in the possibility of developing a process for the antibiotics separation and purification that is able to simplify the number of stages of the

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actual production process, thus minimizing the economical cost of industrial production and reducing the risk of environmental impact by eliminating the use of organic solvents, besides increasing the quality of the extracted product [5,6]. This aspect is of vital important if we think about the nature of the product and its use by humans.

Obviously, the first step in order to evaluate the possibilities of the supercritical extraction as an alternative process for the extraction of liquid solvents is to determine the solubility of the penicillins in a supercritical solvent and its variation under pressure and temperature.

The work was divided into two parts: (a) the experimental determination of the solubility data; the Penicillin G in supercritical carbon dioxide in a pressure range from 100 to 350 bar and in a temperature range from 313.15 to 333.15 K; and (b) the elaboration of two models; one of them based on an empirical equation, the other one based on thermodynamics aspects and the use of equations of state such as Redlich–Kwong and Soave–Redlich–Kwong, for the correlation and prediction of the solubility of Penicillin G in supercritical carbon dioxide and its variation under the experimental conditions.

2. Material and methods

The apparatus used in this work was an 'Autoclave Engineers' supercritical fluid extraction (model 'SCE Screening System') modified, according to those used by several other investigators, in order to carry out the present work. The flow diagram of the installation is shown in Fig. 1, and the apparatus and its principle applications are described below.

The liquid solvent, carbon dioxide, was cooled to prevent its gasification and introduced into a Milton Roy HPLC pump of 46–460 ml h^{-1} capacity with a cooling head. Liquid CO_2 was delivered by the pump through a heating coil so that the CO_2 could attain the desired operation temperature prior to entering the equilibrium cell. The pump was switched on and its output pressure increased by the pressure regulating valve (PRV) so as to reach the required processing pressure.

The equilibrium cell, constructed from stainless steel tubing was 40 cm long with 0.365 cm O.D. and was packed with alternate layers of the antibiotic and glass beads (siran™) with a heating jacket through which heating water was passed in order to achieve the temperature process. Constant temperature was maintained with a water bath. Flow control was maintained with a flow metering valve (FMV). The dissolved antibiotic in the supercritical CO_2 was separated from the carbon dioxide and collected in two cold traps containing methanol. The CO_2 was passed through a volumetric flow meter (Bronkhorst High-tech B.V., F-111C).

An absorption spectroscopic method using a UV-spectrometer 'Shimadzu' (model 'Multipurpose Recording Spectrophotometer' MPS-2000) was used to analyze the penicillin–methanol sample solution, which contains a sharp absorption maximum at 203 nm in the ultraviolet band. Calibrations were made using standard solutions of Penicillin G in methanol at different levels of concentrations.

The Penicillin G was supplied by the Antibióticos, S.A. Company and the carbon dioxide was supplied by Carburos Metálicos, S.A. and was of a minimum purity of 99%.

To check that the solvent flow was adequate to keep the CO_2 saturated in Penicillin G, the flow range where the solubility did not change was determined. This study was made under all the experimental conditions, i.e. at 200 bar and 40°C (Fig. 2 shows this effect).

As can be seen, the solubility of Penicillin G does not change over the range 0.5–1.5 ml min^{-1}. Above this range, it is not possible to operate because the system cannot reach equilibrium; below 0.5 ml min^{-1} is not possible either because it would imply a difficult quantification of the total amount of solvent.

3. Results and discussion

3.1. Solubility data

The experimental solubility data for Penicillin G (as the molar fraction) in supercritical carbon

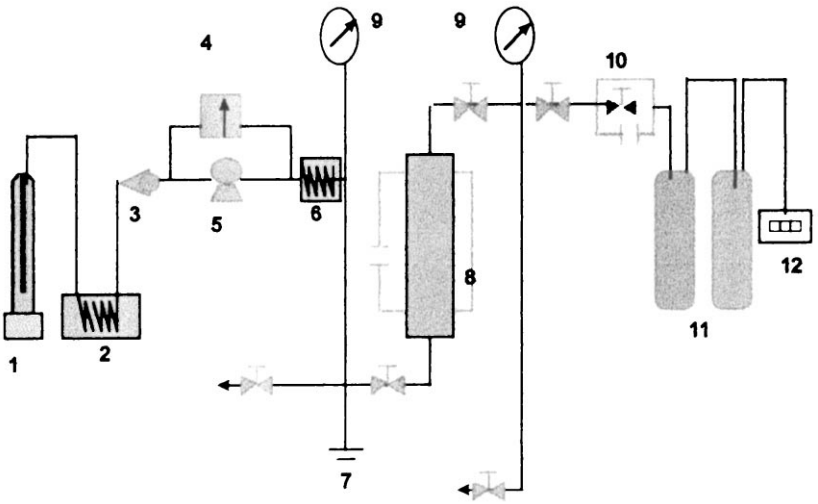

Fig. 1. Schematic diagram of the experimental apparatus: (1) gas tank; (2) constant temperature bath; (3) check valve; (4) pressure regulating valve; (5) liquid pump; (6) preheater; (7) rupture disc; (8) equilibrium cell; (9) pressure gauge; (10) metering valve; (11) cold traps; (12) volumetric flow meter.

dioxide is shown in Table 1 (reproducibility $\sim 1.1 \times 10^{-6}$ molar fraction). From the obtained results, it can be seen that Penicillin G solubility always increases as the pressure does; this is because the CO_2 density increases, so the solvent power does also. This effect occurs because of the decrease in intermolecular distance, so the solute–solvent interactions increase [7].

The solubility behavior with temperature is more complex. At pressures over 150 bar, the solubility increases with increasing temperature, but at 100 bar, an opposite effect happens. The two predominant influences on the solubility are the solute vapor pressure and the solvent density. As the temperature increases, the vapor pressure increases too, which enhances the solubility; but the density and the CO_2 solvent power decrease, which makes the solubility decrease.

At lower pressures, the effect of the density is more important, so the solubility decreases. For pressures above 150 bar, the solubility increases with increasing temperature because the decrease in CO_2 density cannot overcome the increase in vapor pressure.

This solubility behavior agrees with others obtained by different authors for different compounds: Penicillin V (similar characteristics to

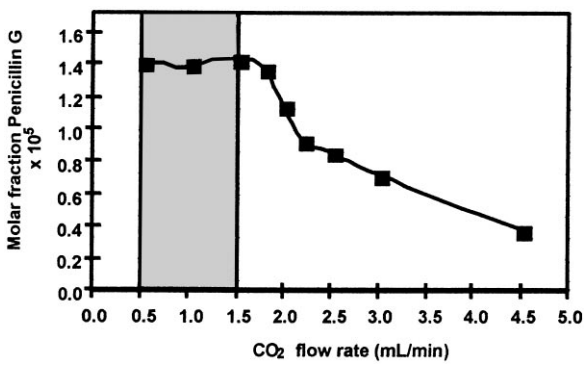

Fig. 2. Effect of carbon dioxide flow rate on the solubility determination.

Table 1
Solubility of Penicillin G in supercritical carbon dioxide (molar fraction $\times 10^5$)

Pressure (bar)	313.15 K	323.15 K	333.15 K
100	0.535	0.462	0.420
150	0.485	0.613	0.849
200	1.43	1.82	2.39
250	1.85	2.70	3.89
300	2.00	3.87	5.29
350	1.98	4.62	6.33

Penicillin G) [1]; glucose and fructose [8]; progesterone, testosterone and cholesterol [9].

3.2. Data correlation

There are two common approaches to correlate and predict solubility, namely empirical and semiempirical models.

To correlate and predict solubility from semiempirical models by using equations of state requires tedious computational effort and physical property values that are often difficult to obtain. Therefore, it is often easier to predict solubility in the limited pressure and temperature range of measurement by using an empirical model.

The error of the fit was determined by calculating the average absolute relative deviation (AARD) between the measured and calculated data, defined as:

$$\mathrm{AARD} = \frac{1}{N}\sum_{i=1}^{N}\left|\frac{y_{\mathrm{exp}} - y_{\mathrm{cal}}}{y_{\mathrm{exp}}}\right| \times 100 \qquad (1)$$

3.2.1. Empirical models

- The Chrastil [10] equation relates the solubility of the solute (c, g l^{-1}) to the density (ρ, g ml^{-1}) and temperature (T, K), as given below:

$$\ln c = c_0 + \frac{c_1}{T} + c_2 \ln \rho \qquad (2)$$

- Del Valle and Aguilera [11] modified the above equation by including an additional second order temperature term:

$$\ln c = c_0 + \frac{c_1}{T} + c_2 \ln \rho + \frac{c_3}{T^2} \qquad (3)$$

- Yu et al. [12] proposed an equation which relates the solubility of the solute (y, molar fraction) to the pressure (P, bar) and the temperature (T, K):

$$y = c_0 + c_1 P + c_2 P^2 + c_3 PT(1-y) + c_4 T + c_5 T^2 \qquad (4)$$

As can be seen in Table 2, correlation of the experimental data using equations gave poor results.

In order to improve these results, a new empirical model was proposed:

$$\ln y = c_0 + c_1 P + c_2 P^2 + c_3 PT + c_4 T + c_5 T^2 \qquad (5)$$

which related the solubility of Penicillin G (y, molar fraction) to the pressure (P, bar) and the temperature (T, °C). The results are presented in Fig. 3 and Table 2.

The predicted solubilities were in good agreement with the experimental data for Penicillin G.

3.2.2. Semiempirical models

The molar fraction of Penicillin G in the supercritical fluid (y_2) has been calculated from its thermodynamic definition (Table 3), where P_2^{sat} and v_2^{s} are the vapor pressure and the molar volume of pure solid, and $\hat{\phi}_2^{\mathrm{F}}$ is the fugacity coefficient of component 2 in the supercritical phase (at pressure P and temperature T). The calculation of the solubility y_2, therefore requires a knowledge of P_2^{sat}, v_2^{s} and an equation of state (with its associated mixing rule) for the estimation of $\hat{\phi}_2^{\mathrm{F}}$.

The Ambrose group contribution method [13] was used to calculate the critical pressure and molar volume, and the boiling temperature of the Penicillin G. T_{c} was estimated by the Fedor group

Table 2
Calculated regression parameters and deviations for Eqs. (2)–(5) for Penicillin G in supercritical carbon dioxide

Model	C_0	C_1	C_2	C_3	C_4	C_5	AARD (%)
Chrastil [10]	10.8	−5904	2.67	–	–	–	32.4
Del Valle and Aguilera [11]	−0.28	1272	2.67	−1158320	–	–	32.4
Yu et al. [12] ($\times 10^6$)	−380	−2.76	3.95×10^{-5}	3.42	-7.0×10^{-3}	9.0×10^{-3}	22.9
Proposed ($\times 10^2$)	−1464	1.05	3.62	0.024	-3.0×10^{-3}	0.06	14.4

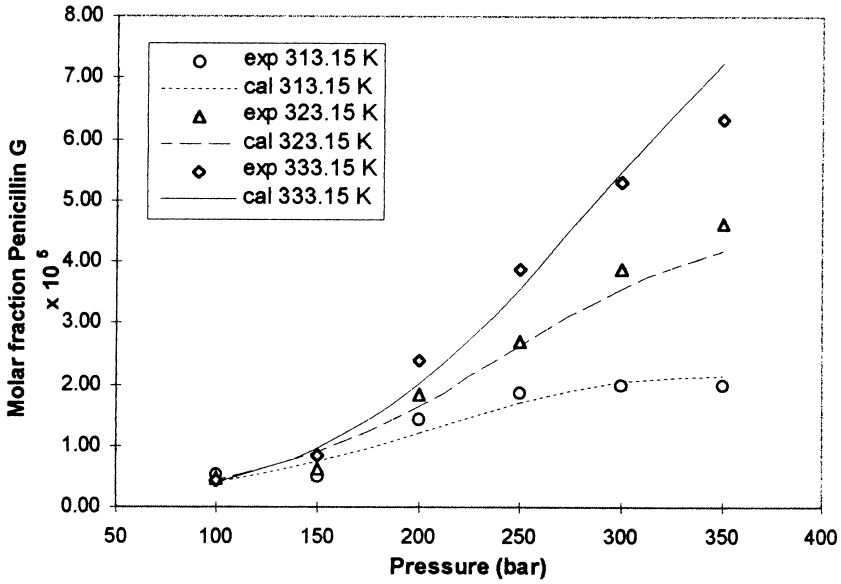

Fig. 3. Experimental and calculated solubility by using Eq. (5) at different temperature versus pressure.

contribution method [13]. The critical properties obtained in this way are not true critical properties, but rather pseudocritical parameters which allow the equation of state to be used.

For every component i in the mixture, the fugacity coefficient [14] is given by the relation that is described in Table 3, where z is the compressibility factor and n_i is the mole number of component i.

In this work, the Redlich–Kwong [15] (RK) and Soave–Redlich–Kwong [16] (SRK) equations of state with Lorentz–Berthelot [17] mixing rules were used to calculate the fugacity coefficient

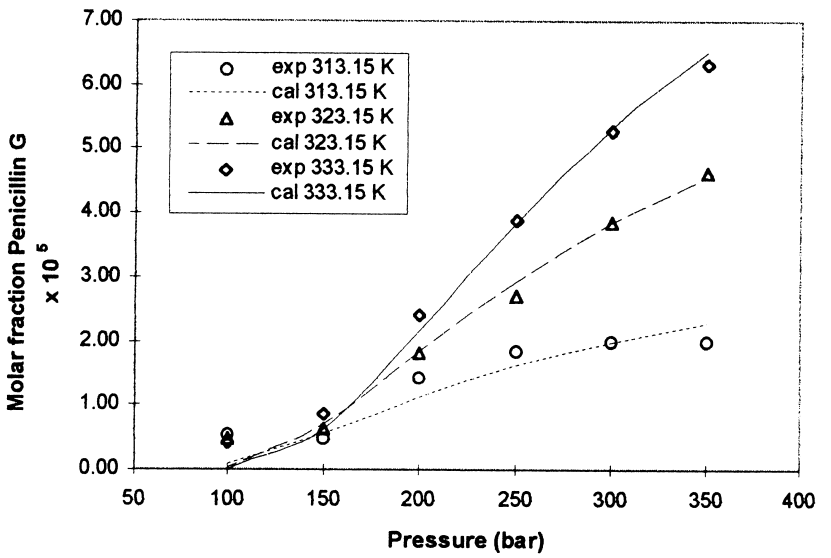

Fig. 4. Data correlation using the Redlich–Kwong equation of state.

Table 3
Equations of state for mixtures using Lorent–Berthelot mixing rules

Fluid–solid equilibrium

$$y_2 = \frac{P_2^{sat}}{\hat{\phi}_2^F P} \exp \frac{(P - P_2^{sat}) v_2^s}{RT}$$

Estimated physical properties of Penicillin G

$P_c = 23.55$ bar	estimated by Ambrose's method
$V_c = 0.940$ l mol^{-1}	estimated by Ambrose's method
$T_b = 715.02$ K	estimated by Ambrose's method
$T_c = 902.78$ K	estimated by Fedor's method
$\omega = 1.3249$	estimated by the Lee–Kesler correlation

Redlich–Kwong equation of state

$$P = \frac{RT}{v-b} - \frac{a\alpha}{v(v+b)}$$

$$\alpha = \frac{1}{\sqrt{T}}$$

Soave–Redlich–Kwong equation of state

$$P = \frac{RT}{v-b} - \frac{a\alpha}{v(v+b)}$$

$$\alpha = \left[1 + s\left(1 - \sqrt{\frac{T}{T_c}}\right)\right]^2$$

$$s = 0.48508 + 1.55171 \times \omega - 0.15613 \times \omega^2$$

Fugacity coefficient

$$\ln \hat{\phi}_i = \int_0^P \left[\left(\frac{\partial z}{\partial n_i}\right)_{P,T,n_{j\neq i}} - 1\right] \frac{dP}{p}$$

Lorent–Berthelot mixing rules

$$a = \sum_i \sum_j y_i y_j a_{ij}$$

$$a_{ij} = 0.42748 \frac{R^2 T_{cij}^{2.5}}{P_{cij}} \text{ (RK)}$$

$$T_{cij} = (1 - K_{ij}) \sqrt{T_{ci} T_{cj}}$$

$$V_{cij} = \left(\frac{V_{ci}^{1/3} + V_{cj}^{1/3}}{2}\right)^3$$

$$b = \sum_i y_i b_i$$

$$a_{ij} = 0.42748 \frac{R^2 T_{cij}^{2.5}}{P_{cij}} \text{ (SRK)}$$

$$Z_{cij} = \frac{Z_{ci} + Z_{cj}}{2}$$

$$P_{cij} = \frac{Z_{cij} R T_{cij}}{V_{cij}}$$

(Table 3). In order to calculate this coefficient, a binary interaction parameter, K_{ij} must be obtained by fitting the experimental solubility data. The acentric factor (ω) was calculated by the Lee–Kesler correlation [18].

As regards molar volume calculation, experimental determination of solid density as well as group contribution methods are available. In this work, the solid density was determined using a mercury porosimeter (Carlo Ebra, Pascal 140). The obtained molar volume was 0.226 l mol^{-1}.

For this system, two parameters, K_{12} and the vapor pressure (P_2^{sat}) of Penicillin G, were estimated by minimizing the error (AARD) between experimental and predicted solubility data.

Table 4 presents the results; the predicted vapor pressure and binary interaction parameter by regressing the experimental data with Redlich–Kwong and Soave–Redlich–Kwong equations of state.

As could be expected, the obtained vapor pres-

Table 4
Regressed binary interaction coefficients and P_2^{sat} for RK and SRK equations of state for the carbon dioxide–Penicillin G system

	T (K)	K_{12}	P_2^{sat} (bar)	AARD (%)
RK	313.15	0.003	3.55×10^{-12}	23
	323.15	0.001	2.24×10^{-11}	23
	333.15	0.007	1.44×10^{-10}	23
SRK	313.15	0.319	2.82×10^{-12}	21
	323.15	0.331	4.57×10^{-11}	21
	333.15	0.366	3.09×10^{-9}	21

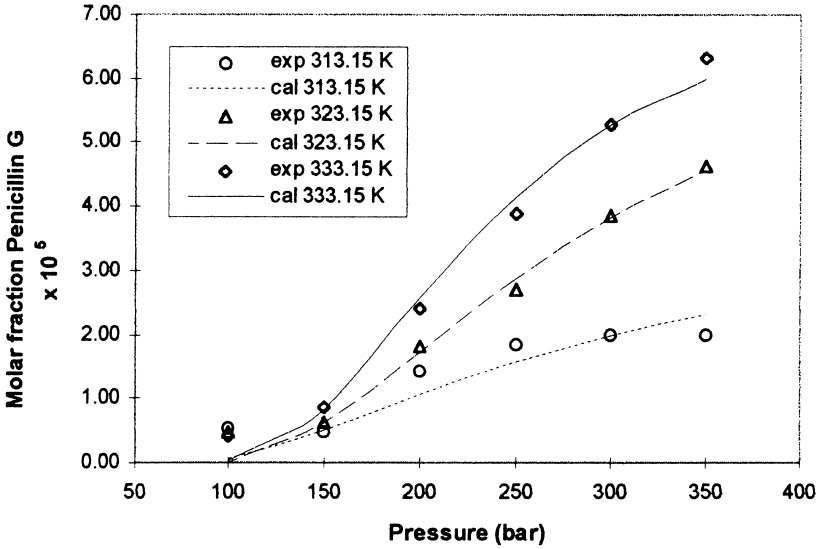

Fig. 5. Data correlation using the Soave–Redlich–Kwong equation of state.

sure data increased as the temperature did. The obtained results in the solubility prediction show that the correlation with the Soave–Redlich–Kwong equation (AARD=21%) gives better results than with the Redlich–Kwong equation (AARD=23%). In both cases, the agreement between calculated and experimental data can be considered very good, as can be seen in Figs. 4 and 5.

This model was particularly poor in the low pressure region, with an AARD of 93%. At high pressures, where the supercritical extraction is more important because of the higher solubility data, the AARD was less than 9% using the RK equation and 6% with the SRK equation.

4. Conclusions

The solubility of Penicillin G in supercritical carbon dioxide was measured over an extended range of temperatures and pressures. It can be affirmed that, for pressure above 150 bar, the solubility increases with increasing temperature and pressure. For pressure lower than 150 bar, the solubility increases with increasing pressure and decreases with temperature.

The Redlich–Kwong and Soave–Redlich–Kwong equations of state provide a good prediction for the solid–fluid equilibrium of the penicillin and supercritical CO_2 system. In the same way, the predicted solubilities with the empirical model are in good agreement with the experimental data.

The observed solubility data make the consideration of supercritical fluid extraction possible as a feasible production method in the pharmaceutical industry. However, because of low values of solubility, future research must clarify the possibility of designing a supercritical process by extracting the antibiotic from the fermentation broth.

Moreover, in the conventional process of antibiotic production, traces of the solvent can be left behind in the antibiotic. Supercritical fluid extraction can be used for solvent removal from antibiotic using neat CO_2 [19].

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