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Binding constants of oxytetracycline to animal feed divalent cations

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Abstract

The binding constants of different divalent cations (Ca^{+2}, Mg^{+2}, Fe^{+2}, Hg^{+2}, Ni^{+2} and Co^{+2}) to oxytetracycline (OTC) has been estimated. OTC can be used in veterinary formulations for the prevention and control of disease and added to feed for such a purpose at a dosage rate of 25–700 mg/kg. Feeds contain Ca^{+2} and Mg^{+2} carbonates as raw materials (at a percentage level), and Fe^{+2} and Co^{+2} as additives (at a ppm level), but also can contain Hg^{+2} as a contaminant from fish-based raw materials, or Ni^{+2} as a residual catalyser used in the partial hydrogenation of seed oils. It is key to develop reliable methods for OTC determination, paying special attention to OTC extraction from feed matrices containing those divalent cations, since the particular structure of OTC contains electron-donor groups able to form strong complexes with such metal ions. The values obtained for K_{OTC} show that OTC exhibits a large affinity for Ca^{+2} and Mg^{+2} and decreases on the increase of the charge/ionic radius ratio for the other divalent cations, what means that Ca^{+2} and Mg^{+2} can make more difficult OTC extraction from feeds.

Keywords: Oxytetracycline; Divalent cations; Binding constants; Synchronous spectrofluorimetry

1. Introduction

Oxytetracycline (OTC; Scheme 1) is one of the important members of the tetracycline group of antibiotics which is routinely used in animal husbandry. It is used for the prophylaxis and treatment of a great number of diseases since this antibiotic possesses a broad spectrum activity against many pathogenic organisms. OTC can be used in veterinary formulations for the prevention and control of disease and added to feed for such a purpose. It is licensed for use in a wide variety of food-producing animals such as cattle, pigs, sheep, poultry and it is a principal antibiotic used in fish farming too (Coopper et al., 1998; Farrington, Tarbin, Bygrave, & Shearer, 1991; McCracken, Blanchflower, Haggan, & Kennedy, 1995; Pouliquen, Keita, & Pinault, 1992; Stubbings, Tarbin, & Shearer, 1996). Its usage may result in residues in food products of animal origin, often due to the improper observance of withdrawal periods. For this reason, OTC is often determined in those foods; for example, some authors found this antibiotic in honey (Salinas, Berzas, & Espinosa, 1989; Xie, Dong, Fen, & Liu, 1997), in milk (Boatto, Pau, Palomba, Arenare, & Cerri, 1999; Carson & Breslyn, 1996; Carson, Ngoh, & Hadley, 1998; Furusawa, 1999a; Pena, Lino, & Silveira, 1999; Tjørnelund & Hansen, 1997), in eggs (Coopper et al., 1998; Croubels, Vanoosthuyze, & Van Peteghem, 1997; Furusawa, 1999b, 1999c; Omija, Mitema, & Maitho, 1994), and in different animal tissues (Carson et al., 1998; Coopper et al., 1998; Croubels et al., 1997; De Wasch et al., 1998; Farrington et al., 1991; McCracken et al.,

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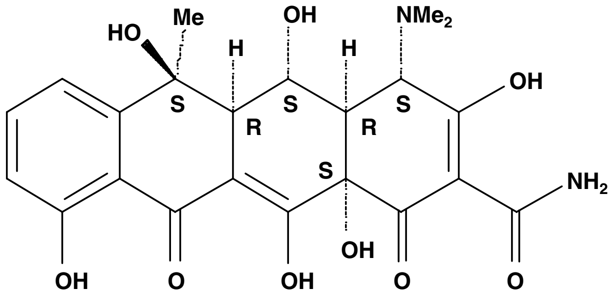

Scheme 1.

1995; Pouliquen et al., 1992; Sokol & Matisova, 1994; Stubbings et al., 1996; Walsh, Walker, & Webber, 1992). OTC has been measured too in other matrices different from food, like plasma (Iwaki, Okumura, & Yamazaki, 1993), urine (Sharma, Koritz, Perkins, & Bevill, 1977; Weimann & Bojesen, 1999; Xie et al., 1997), feeds (Fernández-González, García-Falcón, & Simal-Gándara, 2002; Hasselberger, 1993; Houglum, Larson, Mutchler, & Wetzler, 1998; Markakis, 1996; Martínez & Shimoda, 1988; Weng, Sun, Roets, & Hoogmartens, 2003), premixes and pharmaceutical formulations (Chappell, Houglum, & Kelley, 1986; Croubels et al., 1997; Fernández-González et al., 2002; Houglum et al., 1998; Iwaki, Okumura, & Yamazaki, 1992; Katz & Fassbender, 1973; Kazemifard & Moore, 1997; Weng et al., 2003), etc. In the matrix of the samples analysed exist lots of components that can interfere with OTC determination. In samples like feeds, interfering substances such as pigments, additives, fatty acids and mineral cations occur naturally (Salvatore & Katz, 1993). It is of paramount importance to achieve a selective OTC extraction.

Recently, our research group (Fernández-González et al., 2002) has demonstrated that synchronous spectrofluorimetry is a versatile analytical technique ideal for OTC determination in analytical quality control and for research and development laboratories in the food and beverage industry. This method (Fernández-González et al., 2002) is reliable (with good response linearity, high recovery and precision, and low detection and quantification levels), simple and fast.

The particular structure of this antibiotic, as in the case of other tetracyclines, contains electron-donor groups able to form strong complexes with metal ions (Carson & Breslyn, 1996; Lambs, Decock-Le Révérend, Kozlowski, & Berthon, 1988). Therefore, some authors use different OTC extraction procedures based on liquid–liquid extraction with calcium (Sharma et al., 1977) or clean-up with metal chelate affinity chromatography, which consists in one column preloaded with divalent cations where OTC is specifically adsorbed by chelation with the metal ions (Carson & Breslyn, 1996; Carson et al., 1998; Coopper et al., 1998; Croubels et al., 1997; De Wasch et al., 1998; Farrington et al., 1991; Stubbings et al., 1996). We must also take into consideration that these ions can be naturally present in the samples to be extracted, which makes difficult to extract OTC from animal tissues (Pouliquen et al., 1992; Sokol & Matisova, 1994) or from feeds that contain a large amount of calcium, magnesium and other cations (Fernández-González et al., 2002). In the present paper, the binding constant of different divalent cations (Ca^{+2}, Mg^{+2}, Fe^{+2}, Hg^{+2}, Ni^{+2} and Co^{+2}) to the OTC has been estimated with the intention of providing information about the potential difficulties to extract OTC from divalent cation-containing feeds.

2. Experimental

2.1. Reagents

Oxytetracycline hydrochloride (OTC) was purchased from Riedel-de Haën (Seelze, Germany). Because OTC is not very stable and is susceptible of photochemical and oxidative degradation, work solutions were freshly prepared every day. Divalent metal salts included calcium chloride anhydrous ($CaCl_2$), supplied by Panreac (Barcelona, Spain); nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$) from Riedel-de Haën (Seelze, Germany); cobaltous chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), mercuric chloride anhydrous ($HgCl_2$) and ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), from Fluka (Steinheim, Germany). All solutions were prepared in highly pure water ($R \geqslant 18$ MΩ) obtained from a Millipore water purification system.

2.2. Apparatus and operating conditions

All spectrofluorometric measurements were taking by means of a Jasco P-750 luminescence spectrophotometer equipped with a xenon lamp, Monk-Gillieson monochromators and 1 cm quartz cuvettes. The spectral data were collected and processed using the Spectra Manager software. The optimised instrumental parameters for synchronous scanning of OTC complexes were as in Table 1.

2.3. Procedure

Association constants were measured by spectrofluorometry, using reported procedures in the literature (García-Río, Hervés, Mejuto, Parajó, & Pérez-Juste, 1998), by making use of different synchronous spectra of

Table 1
Optimised instrumental parameters for synchronous scanning of OTC complexes

Parameters	Spectrofluorometric conditions
Spectral range	400–600 nm
Excitation and emission slits	10 and 5 nm respectively
Scan speed	125 nm/min
Excitation-emission wavelength difference (Δλ)	115 nm