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Crystallization of porcine insulin with carbon dioxide as acidifying agent

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ABSTRACT

Recent studies on the use of volatile electrolytes such as CO_2 in protein precipitation showed that these agents are a promising alternative to the conventional acids. This use of volatile electrolytes prevents protein denaturation due to local pH extremes, and saline effluent generation is greatly reduced, as the volatile electrolyte may be separated and recovered from solution just by pressure release. In this work, insulin was successfully crystallized in the presence of zinc using CO_2 as acidifying agent. The crystals obtained were rhombohedral, a common shape for porcine insulin crystals that contain zinc in their structure, and their average size varied with the mixing applied.

1. Introduction

About 70% of the products sold by the process and pharmaceutical industries are solid [1]. In the pharmaceutical industry crystallization is an important technological process for particle formation and over 90% of all pharmaceutical products, such as tablets, aerosols, capsules, and suspensions, generally contain drugs in crystalline form [2]. Protein crystallization is a technique optimized over many decades to produce crystals with better conditions for X-ray diffraction analysis. Its potential in protein recovery and purification has recently been revealed. The crystal form of protein products has an advantage over the dissolved and amorphous forms, since it has longer storage life and higher purity [3].

Frequently isoelectric precipitation is used in downstream processing for separation of proteins from aqueous solutions. This method consists of adjustment of solution pH to the isoelectric point of the protein (pI). At the pI, the molecule carries no net electrical charge, reducing the electrostatic repulsion between molecules which results in precipitation. Since the majority of proteins have a pI in the acid region, conventional acids such as H_2SO_4 and HCl are commonly used for pH adjustment in these precipitations. After precipitation, the effluent generated must be treated before its disposal into the environment, a complex process step that can be costly.

The use of volatile electrolytes such as CO_2 is an alternative to the use of conventional acids in the isoelectric precipitation processes. They are called volatile electrolytes since, as exemplified in the case of CO_2, they dissolve and dissociate in water resulting in ions (carbonate, bicarbonate, and H^+) whose concentrations are dependent on the temperature and pressure of the system, causing a reduction in pH. Under depressurization of the system the anions CO_3^{2-} and HCO_3^- pass from the liquid phase to the vapor phase as CO_2 and can be removed from the system and recycled in the process. Another advantage of the use of volatile electrolytes is that during adjustment of protein solution pH, extremes in local pH are prevented because solubilization of the precipitating agent is slow and more homogeneous, occurring along the whole gas–liquid interface. In conventional processes, these extreme pH values can result in the denaturation of protein or even a reduction in precipitate purity [4].

The use of volatile electrolytes in downstream processes of proteins is relatively recent with only a few reports in the open literature. The published studies on protein precipitation with volatile electrolytes were carried out with complex protein systems such as milk, soy protein extracts [5,6], and the fractionation of protein mixtures [7,8]. The isoelectric precipitation with carbon dioxide of a single protein, bovine serum albumin, was investigated by Qi et al. [9]; additional investigations of the activity of some enzymes in the system formed by ethanol, carbon dioxide, and water were presented by Yao et al. [10]. Samadi and Husson [11] described a process of separation by precipitation with CO_2 for recovering L-aspartic acid. Tashima et al. [12] were pioneers in reporting experimental and theoretical aspects of the precipitation of a single protein (porcine insulin) using CO_2 and developed a thermodynamic model to correlate the experimental data. No crystal formation was reported in that work.

The objective of this work was to find conditions for the crystallization of a single protein using CO_2 as acidifying agent, and we successfully crystallized porcine insulin in the presence of zinc in a pressurized CO_2 stirred tank crystallizer.

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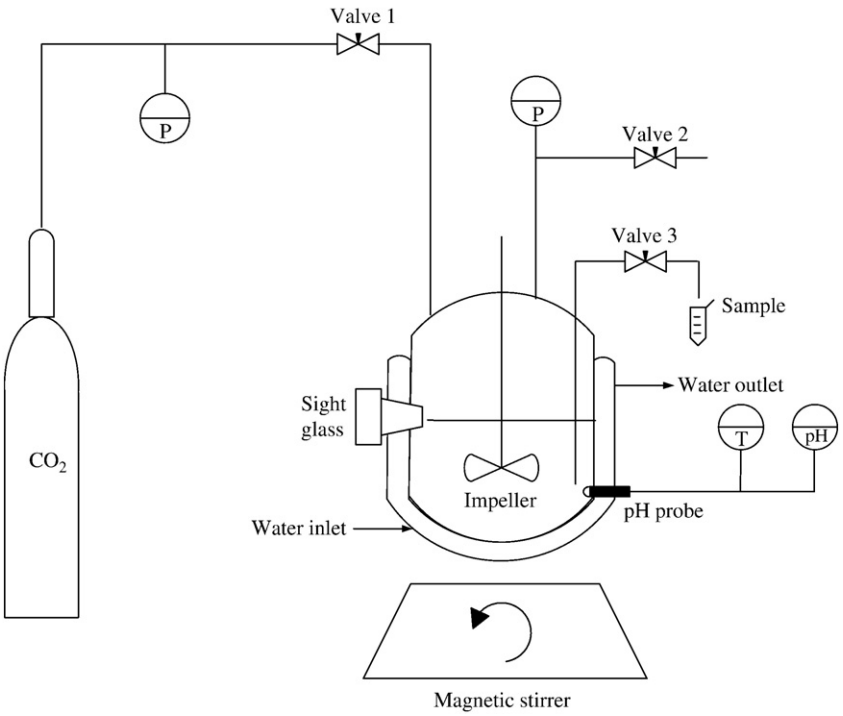

Fig. 1. Scheme of the experimental apparatus for the crystallization of proteins with CO_2.

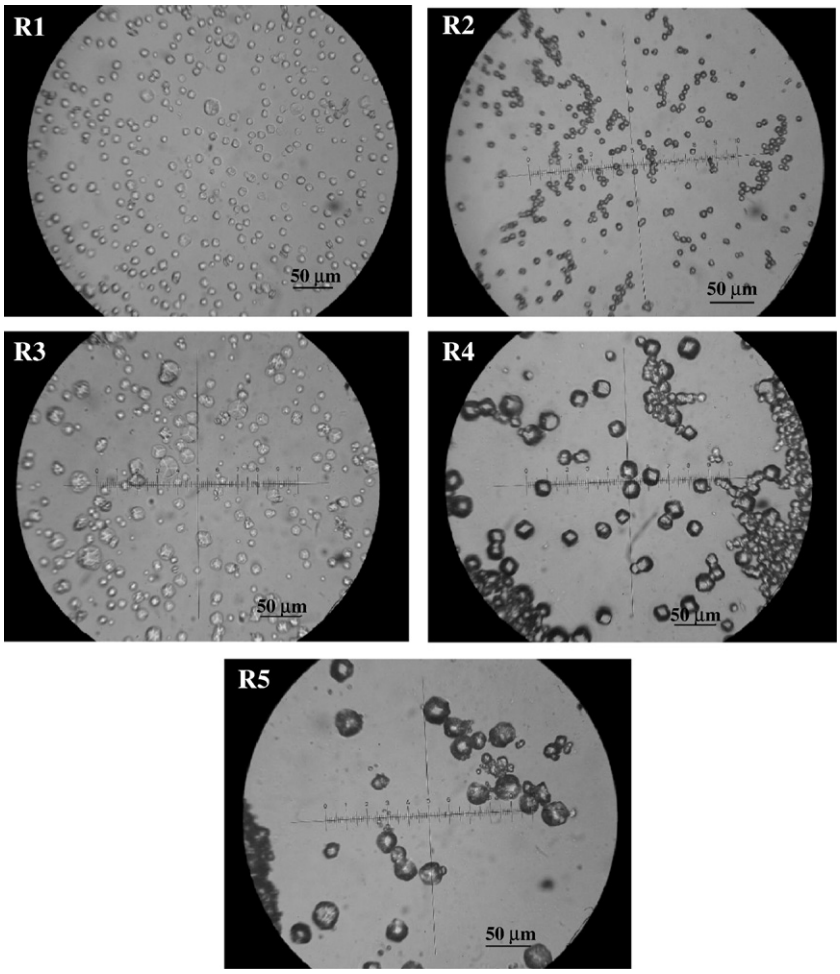

Fig. 2. Photomicrographs of porcine insulin crystals in 50 mM $NaHCO_3$ and 0.4 mM $ZnCl_2$ under pressurized CO_2 at 5 °C. Equilibrium pH runs: R1 (magnetic stirring), pH 6.50; R2 (magnetic stirring), pH 6.50; R3 (impeller stirring), pH 6.37; R4 (impeller stirring), pH 6.35; and R5 (impeller stirring), pH 6.46.