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Crystal growth of inorganic, organic, and biological macromolecules in gels

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"Nowadays, all we know is that Nature takes thousands and thousands of years to obtain beautiful minerals (gems) like sapphires, rubies, diamonds, among others that have made people crazy due to their beauty, but the gel method was born to allow us to play with instantaneous mineralogy" H.K. Henisch 1988 [1].

1. Historical data on the gel method

The pioneering experiment related to crystal growth in gels was performed in 1896 by Liesegang, who obtained periodic precipitation of inorganic compounds grown in gelatine [2]. These rings were considered of interest, partly due to their obscure origin and partly because they were reminiscences of certain structures found in nature [2]. A year later, in 1897, Wilhelm Ostwald was also the first to propose an explanation to the phenomenon observed by Liesegang [3]. Ostwald's hypothesis was based on the propagation of a supersaturation wave; this suggestion was matured by Ostwald up to the middle of the 1920's [4]. At that time, there had already been scientific contributions that attempted different crystal growth experiments such as ice crystals grown on ice cream, Rochelle's salt grown in cheese, and crystals of sulphur grown in rubber [5]. There were some publications even on the crystallisation of uric acid in joints [6]. However, all these experiments were inconsistent according to the principles of crystals growth in gels in terms of reproducibility. The gel method was neglected just until the beginning of the 50's of the twentieth century, when a PhD student from Harvard University (Lewin, 1950) first attempted to crystallize biological macromolecules in gels. At that time, most geologists thought that all quartz on earth had once been silica hydrogels. The most remarkable experiment closely related to the crystal growth in gels was performed by Eitel in 1954 [7]. According to historical records, microscopic crystals of silica were obtained from silica gels in the presence of various "crystallisation agents" when heated in water vapour under certain pressure. In 1954 Low and Richards reported, for the first time, the crystallisation of albumin in gelatine (based on the idea of Lewin [8]. Then, there was, once again, a gap (with very few reports of the use of gel growth method), up to 1975, when organic gels were introduced for biological crystallisation in a short review published by Dondi [9]. He applied the gel method for electrophoresis in the crystallisation of ribosomes in order to crystallize these large biomolecules in situ. During the 70's and the 80's all information related to the gel growth method was summarized in the book published by Prof. Henisch in 1986, including some chapters related to theories of crystal growth and transport processes that were co-signed with Prof. García-Ruiz [10,11]. This book inspired many scientists to grow crystals (mostly minerals, but also some organics) in different types of hydrogels and a variety of experimental setups (see Figs. 1 and 2). The French group focused on mineral growth led by Lefacheux who introduced in 1988 the use of specific silica gels (tetramethyl orthosilicates) in the crystallisation of proteins and inorganics. Even this group, suggested agarose as one of the easy-to-use gels for the growth of crystals of any kind [12].

At the beginning of the 90's in the twentieth century, the group of Prof. García-Ruiz, in Spain, pioneered a theory involving molecular simulations of crystal growth in gels as diffusing-reacting systems [13]. He also proposed to use gel media to crystallize inorganics and biological macromolecules (proteins, nucleic acids and polysaccharides) by using the gel-growth approach [14]. Another approach closely related to crystal growth in gels includes the possibility of crystallising proteins in capillaries (with a diameter less than 0.3 mm). A development of the gel-growth technique involving the role of reducing convective transport as occurs during crystal-growth in capillaries or in microgravity experiments are closely related strategies for the production of high-quality single crystals. Additional consecutive advantages of minimising convective transport has been demonstrated using the capillary tube method [15]. These advances in the growth of proteins in capillary tubes permitted Prof. García-Ruiz and his team to develop, in 1993, the first variant of the counter-diffusion methods, called the gel acupuncture technique (well-known with the acronym GAME) [16]. This novel technique consists of a precipitating agent that diffuses through the gel inside a capillary tube filled with a protein solution, thus also enabling crystallisation of small molecular weight compounds [17]. These methods applied to proteins will be discussed at the end of this review.

Nowadays, the gel growth technique (Fig. 1) has undergone some innovations that includes the use of *ad hoc* devices (Fig. 2) used to grow different types of crystals. This technique has been categorized as part of counter-diffusion methods thanks to the assessment of the different types of gels, capillaries, additives, and different types of crystal growth devices used for the crystallisation of different compounds from small

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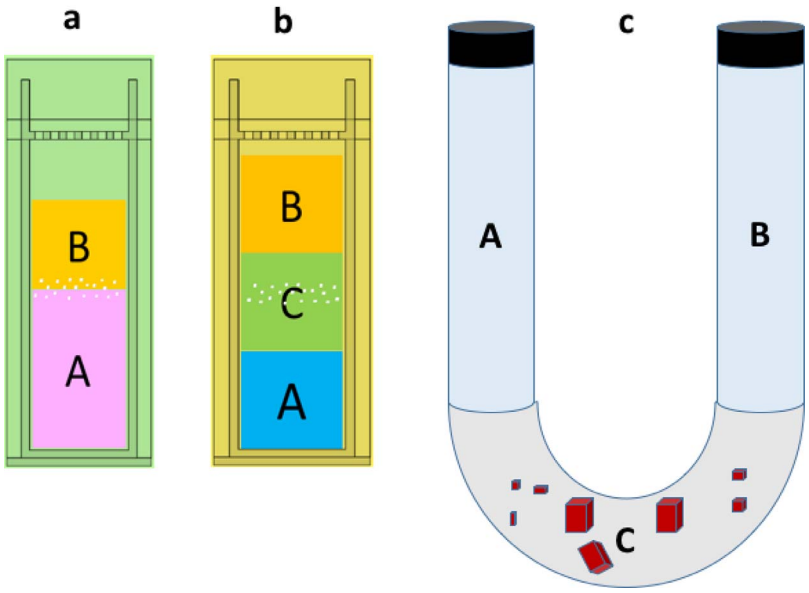

Fig. 1. Basic procedures to grow crystals in gels. a) method two layers, b) method three layers, c) U-tube method.

to biomacromolecular crystals [18]. In contrast to other techniques using capillaries, different levels of supersaturation can exist, allowing the precipitation to occur in very high supersaturation zones (nucleation occurs when supersaturation is high, and growth occurs as supersaturation diminishes). Several authors have demonstrated that gel growth increases the quality of crystals as compared to solution growth as well as demonstrating the probability of finding optimal crystallisation conditions (see for instance reference [15]).

So far, we have generally mentioned the advantages of the gel growth method of inorganics, organics and soluble proteins. However, there are also some strategies and approaches known as *in meso* crystallisation techniques applied to membrane proteins, (including crystallisation in lipidic cubic and sponge phases) [19]. These techniques have allowed us to obtain over 250 structures of membrane proteins (of these 250 structures over 80 represent unique proteins [20]). The *in meso* techniques could be considered as one of the approaches to crystal growth in gels, they use lipidic or sponge phases instead of gel network. This latter assumption is due to fact that the principles of diffusing-reacting systems and diffusion transport control are perfectly applicable to this *in meso* techniques [21,22].

Some reviews recently published have demonstrated the potential of the gel-method for producing high-quality single crystals of a variety of compounds and biomolecules, compared to crystal growth in solution [23–25]. This gel method was already known at the end of the nineteenth century, however, it had not been widely used to grow crystals from many different compounds until now.

2. Type of gel used to grow crystals: synthesis and structure

Several types of gels have been used for the crystallisation of different types of molecules. The structure of gels where atoms or molecules can diffuse, favours the preparation of good quality crystals. The solubility and the pore size need special conditions of pH and temperature. These determine the type of gel to be used for a certain type of sample. The materials used in crystal growth include agar, silicates, oleates and even fruit jelly [26]. In recent experiments poly(ethylene) oxide has also been used for the crystallisation of inorganic and organic molecules in non-aqueous solvents [27].

The great majority of reports describing crystallisation of different compounds in gels, have used either agarose or silicate hydrogels.

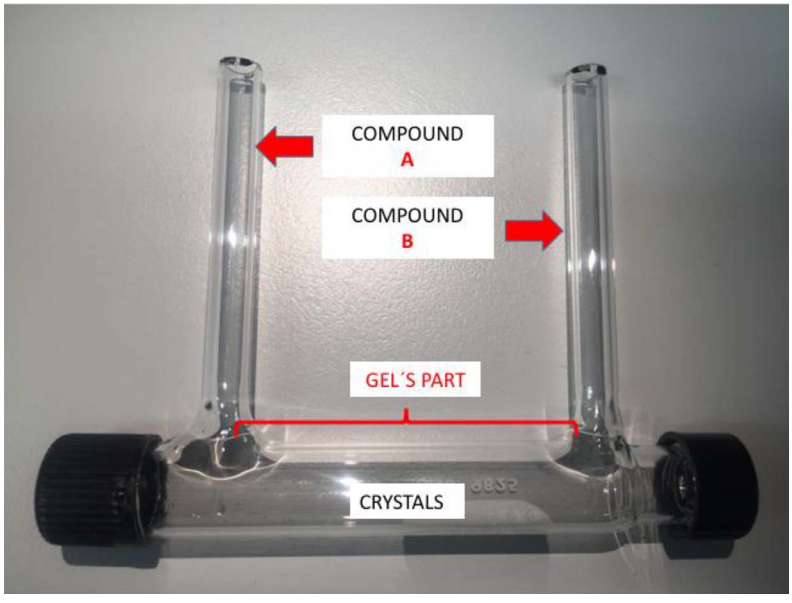

Fig. 2. Device based on the U-tube method. This method allows compounds A and B to counter-diffuse and obtain crystals in the central part.