

## A Refined Biocrystallization Method applied in a Pictomorphological Investigation of a Polymer

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### *Summary*

An experimentally refined biocrystallization method is presented. The method is applicable for investigating the pictomorphological properties of biological samples, as expressed in morphological features of crystal textures. The method is applied in medical research, and in agricultural research concerning crop quality, as a complement to chemical analyses of single compounds. The objective of the present study was to refine existing crystallization chamber techniques through control of physical factors influencing evaporation and crystallization. The reproducibility of the method was studied in a two-fold manner: 1) Concerning the control of physical factors variations in air temperature and humidity were studied during three similar experiments with experimental periods of 17 hours. 2) Concerning the reproducibility of textural features three investigators performed a visual classification of 32 coded crystallograms produced during the experiments, relative to a reference set of seven classes. The crystallograms were produced on the basis of aqueous solutions of the synthetic polymer PVP (Polyvinylpyrrolidone), from different polymerization and concentration levels. Results indicated markedly improved control of air temperature and humidity conditions, relative to a previous study. Results indicated relatively high correct classification scores for all three investigators (69-75%). The correlation coefficients for the 32 observations were high ( $r = 0.91-0.93$ ). Furthermore results indicated a strong correlation between polymerization level of PVP and morphological features.

### *Zusammenfassung*

In der vorliegenden Arbeit wird eine verbesserte Biokristallisationsmethode präsentiert. Die Methode kann für die Untersuchung der pictomorphologischen Eigenschaften, d.h. der morphologischen Charakteristika von Kristalltexturen, von biologischen Proben angewendet werden. Die Methode wird in der medizinischen Forschung und in Ergänzung zu chemischen Analysen in der landwirtschaftlichen Forschung zur Qualitätsprüfung angewendet. Das Ziel der vorliegenden Untersuchung lag in der Verbesserung existierender Kristallisationskammern durch Kontrolle physikalischer Faktoren, welche die Evaporation und Kristallisation beeinflussen können. Die Reproduzierbarkeit der Methode wurde zweifach untersucht: 1) Die Kontrolle physikalischer Faktoren wurde durch Variationen der Temperatur und Luftfeuchtigkeit in drei vergleichbaren Versuchen untersucht, die über 17 Stunden verliefen. 2) Die Reproduzierbarkeit der Kristalltexturen wurde durch Klassifikationen von 32 kodierten Kristallogrammen anhand von Referenzkristallogrammen in sieben Klassen unter-

sucht. Die Klassifikation wurde von drei Personen vorgenommen. Die Kristallogramme wurden unter Zuhilfenahme unterschiedlicher Polymerisierungsraten und Konzentrationen von wässrigen PVP-Lösungen (Polyvinylpyrrolidon) hergestellt. Im Vergleich zu einem früheren Experiment konnte die Kontrolle der Temperatur und Luftfeuchtigkeit wesentlich verbessert werden. Die Ergebnisse zeigten eine relativ hohe positive Bestimmungsrate der Klassifikationen (69-75%) für die drei Personen. Die Korrelationskoeffizienten dieser Klassifikationen waren hoch ( $r = 0.91-0.93$ ). Die Resultate wiesen ferner auf eine starke Beziehung der PVP-Polymerisierungsrate und der morphologischen Charakteristika der Kristalltextur hin.

### *Introduction*

The biocrystallization method, also termed sensitive crystallization and copper chloride crystallization, was originally introduced by *Pfeiffer (1930)*. The term biocrystallization was introduced by *Engqvist (1970)*. The method is applied primarily in medical and agricultural research. A favoured field of application in agricultural research are comparative studies of the effects of different farming systems on crop quality, as a complement to chemical analyses of primary compounds such as minerals, vitamins etc. (*Engqvist 1989; Balzer 1993*).

The method is based on the crystallographic phenomenon that when adding specific inorganic ionic substances, and generally all organic substances, to an aqueous solution of dihydrate  $\text{CuCl}_2$ , crystallograms with reproducible dendritic textures are formed during crystallization (*Kleber & Steinike-Hartung 1959*). Crystallograms exhibit a variety of macro- and microscopical morphological features reflecting the specific admixed sample. In crystallograms produced on the basis of biological samples the individual crystals are integrated into an overall pattern, distributed over the circular crystallization underlay, as opposed to only a peripheral distribution of crystals when crystallizing a pure aqueous solution of dihydrate  $\text{CuCl}_2$ , see Figures 1-3. Hence the compounds and substances are said to have pictomorphological properties. Among numerous organic compounds examined proteins exhibit unique pictomorphological properties (*Neuhaus 1957*). When adding a small amount of human blood (0.2%), changes in crystal structure and colour of the dendrites, as well as chemisorption of nitrogen, carbon and oxygen atoms are observed (*Shibata et al. 1994*).

Different types of textural features can be described by means of morphological terms analogous to those applied in plant morphology. When comparing crystallograms these are traditionally classified visually by experienced investigators, on the basis of textural features. The concentrations of dihydrate  $\text{CuCl}_2$  and the individual sample in question are determined empirically on the basis of initial concentration series. The effects of additives on morphological features are not a monocausal phenomenon, but a complex interaction involving chemical structure and probably hydrophilic properties of the additives, or colloidal properties of the solution (*Barth 1997*).

Reproducibility of a crystallization process presupposes control of physical factors such as air temperature, humidity, levelling of crystallization underlay, vibrations and particles. A broad variety of crystallization facilities has been designed to