

Gelatin layers for holographic purposes: an X-ray diffraction study

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In order to overcome uniformity problems in large u.v. holoconcentrators recorded in dichromated gelatin, the structure of hardened gelatin layers has been studied. X-ray diffraction patterns show that layers are formed by individual gelatin chains partly associated as triple-stranded helical rods as those found in native collagen. These rods, in turn, may form fibrillar aggregates. Helical rods and microfibrils are, to a great extent, parallel to the layer surface and their relative amount and packing depend on the hardener as well as on temperature and drying time after coating. X-ray diffraction offers the possibility of estimating in a simple manner the relative amount of triple-helical rods in crystallographic register within the gelatin layers. From these results, a method for obtaining uniform large holograms by means of a slow layer drying process is proposed.

1. Introduction

From the early days of holography the possibility of replacing some conventional optical systems with holograms has been considered. Holographic elements can be designed to transform any wavefront into any other, and they are lightweight and compact compared with the corresponding conventional elements. However, since they operate by diffraction instead of refraction or reflection, the strong chromatic dispersion limits their use to some specific applications. We have recently designed dichromated gelatin holographic mirrors for solar u.v. energy concentration [1]. These concentrators are intended to supply the activation energy needed in some catalytic photochemical processes by suppressing the longwave solar radiation that reduces efficiency. The reproducibility and uniformity of the spectral characteristics, operating wavelength and bandwidth, of holographic mirrors mainly depend on the nature of the gelatin layer in which they are recorded. In our case, differences in spectral response between the borders and the centre of holograms have been found. The gelatin at the edges behaves as if it were less resistant to processing conditions than that in the centre. Differences are noticeable because of the large 30 × 30 cm dimensions of our holograms, and also because of the vigorous processing required to obtain a large bandwidth. For these conditions, it is observed that, at the borders, the spectral response is shifted to longer wavelengths and, as a consequence, the bandwidth increases considerably.

Gelatin is a well known biopolymer derived by hydrolytic degradation of collagen. The basic molecular unit of collagen is the tropocollagen rod,

a triple-helical structure composed of three separate polypeptide chains. Gelatin dissolves in warm water (> 35 °C) and above this temperature the polypeptide chains exist as flexible single coils. It is now generally accepted [2] that on cooling down the gelatin solution, a partial reversion to ordered collagen-like sequences occurs. It means that during this sol-gel transition a number of gelatin chains are coiled to each other, forming segments of triple-stranded helix separated along the chain by other segments in the disordered random coil conformation. The nature of these junction zones or physical cross-links is quasi-crystalline, although whether these microcrystallites appear during the transition or on the subsequent drying is still under discussion [3,4]. When the gelatin is dried, there is a considerable reduction in volume and the initially isotropic gel structure is compressed into the final dried film. The crystallites appear, then, in a planar orientation and also a certain stretching of the randomly oriented single chains is due to occur [5]. The sol-gel transition so far described is thermoreversible, physical cross-links being easily removed by appropriate changes in physical conditions (heat or water). Hardening is related to the covalent cross-linking, favoured by a hardener agent, which permanently links two or more gelatin molecules giving, as a result, a fixation of the microstructure. In our laboratory we have used chrome-alum as hardener agent. Since optical information is stored in a dichromated gelatin hologram by hardening differential, the initial hardening of the gelatin layer is an important parameter of the process. Hardening and drying processes are superimposed, making this effect more

noticeable at the borders because drying takes place from the edges towards the centre as if it were a big drop of water. For this reason, and because cross-linking by chrome-alum is a slow process, hardening is higher in the centre than at the borders of the layer. Consequently, the structure is different and relatively weak at the borders to justify the lack of uniformity found in holoconcentrators.

In order to improve layer characteristics we have studied the structure of hardened gelatin layers for different conditions of gelation and drying by wide-angle X-ray scattering (WAXS). We have employed this technique following Pezron *et al.* [6] who demonstrated that WAXS is a suitable tool to investigate gelatin gel structure variations. In the first part of this paper the conventional procedure for preparing gelatin gels is described. Next, the structure of a hardened gelatin layer is discussed from X-ray fibre diagrams. The third part shows the influence, on X-ray diagrams, of temperature and drying time during layer preparation. An optimized preparation procedure for gelatin layers is finally proposed and the uniformity of solar u.v. holoconcentrators prepared from these layers is examined.

2. Experimental procedure

2.1. Gelatin layer preparation

We prepared 30 × 30 cm hardened gelatin layers, using chrome-alum as a hardener agent, through the following gravity coating method: (1) Swell 3% by weight of Rousselot gelatin RS-13311 in distilled water for 12 h at 20 °C (The Rousselot gelatin RS-13311 is a photographic-grade alkaline gelatin with an iso-electric point of ca. 5 and a 200 Bloom test.); (2) dissolve gelatin with mild agitation. Increase the temperature at 1 °C min⁻¹ up to 60 °C and then decrease the temperature at the same rate to 50 °C; (3) add 2% chrome-alum by weight of dried gelatin, then decrease the temperature at the previous rate to 45 °C; (4) pipette out and spread solution over 30 × 30 cm levelled glass plates (45 cm³ each); (5) dry at 17 °C and a relative humidity of ca. 30%; (6) store each plate in a thermosealed polyethylene bag at 17 °C and a relative humidity of ca. 30%.

Coating and drying took place in a clean room with controlled temperature and humidity. The thickness of the layers after drying, measured with an interferometry microscope, was 18 μm showing relative thickness variations < 5% and randomly distributed across the layer.

In order to increase the bandwidth of holoconcentrators, double-layer gelatin plates can be prepared as described in one of our previous works [7]. In this case, we successively spread two gelatin solutions with a different amount of chrome-alum each time. The concentration of gelatin solution (2%) and the amount per plate (30 cm³) were adjusted in order to obtain a thickness of 18 μm (9 μm each layer) as in single layers. The lack of thickness uniformity of the double-layers did not increase in comparison with that measured for single layers.

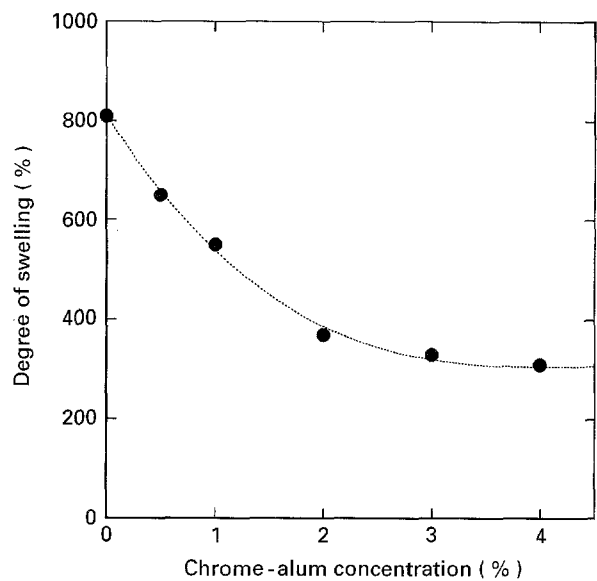


Figure 1 Degree of swell of hardened gelatin layers as a function of chrome-alum concentration.

The hardening of a gelatin layer can be estimated by measuring its degree of swell in a water bath. The highest swelling corresponds to the lowest hardening. Degree of swell is expressed as a percentage increase in layer weight $(W - W_0/W_0)\%$, where W_0 is the weight of the dry layer and W is that of the swollen layer in distilled water at 18 °C for 15 min. Fig. 1 shows the degree of swell of gelatin layers as a function of chrome-alum concentration. We can see that swelling gradually decreases from ca. 800% for non-hardened layers to ca. 370% for layers with a 2% chrome-alum concentration, and then remains nearly constant. We found that gelatins with chrome-alum concentrations < 0.5% were partially dissolved in development baths and led to noisy holograms. On the other hand, for concentrations > 3%, chrome-alum crystals appeared on the layer surface, their number and size increasing for higher concentrations. As a result, we prepared single layers with a 2% chrome-alum concentration and double-layers with 2 and 1.3% chrome-alum concentration for each layer. In addition, some precautions were taken in order to optimize the hardening stability of gelatin layers. It is well known that hardening, in a similar way to a dark reaction, slowly increases at normal room conditions and rapidly when plates are stored at high temperature or high humidity [8]. We observed that gelatin layers stored at 17 °C and a relative humidity of 30% did not show any significant changes after three months.

2.2. X-ray diffraction

2.2.1. Transmission camera

WAXS patterns, with the X-ray beam perpendicular and parallel to the surface of the gelatin samples, were obtained using a point-collimation transmission camera attached to a rotating anode generator with fine focus. The working power was 40 kV × 70 mA and the emission wavelength (Ni-filtered CuK_α) was 0.154 nm. The distance from the sample to the

photographic film was 49.5 mm. Samples of ca. 3×10 mm were obtained from gelatin layers prepared using the method described in the experimental section. The gelatin layer was removed from the glass support to avoid the X-ray scattering from the latter. This operation was facilitated by adding a low concentration (0.2%) of photographic wet agent to the gelatin solutions. In the case of perpendicular incidence of the X-ray beam and since the thin gelatin layer is a poorly crystalline material, the sample thickness was increased by superimposing 16 individual layers. Exposure times up to 20 h were also necessary to record an appreciable diffraction pattern. Similar exposure times were used for parallel incidence which resulted into more intense X-ray patterns. Intensity data in the equatorial and meridional axis of the X-ray photographs were obtained, as optical density, by means of a laser densitometer with a resolution of 0.1 mm on the film surface. Absolute optical density values are affected by different errors which arise from non-linear response of the photographic emulsion for weak signals (optical density < 1).

2.2.2. Diffractometer

We employed a Seifert diffractometer model JSO Debyeflex 2002. The device works with a copper anode with normal focus and a power of $42 \text{ kV} \times 35 \text{ mA}$. Vertical divergence is limited by four slits of 3, 0.5, 1 and 0.2 mm and lateral divergence by two Soller slits collimators with 25 and 50 parallel plates separated 0.5 and 1 mm from each other, respectively. A supportless gelatin sample of 29×39 mm placed onto a stainless steel rectangular-flat holder was used in the experiments. Diffractograms were recorded in the angular region $2^\circ \leq 2\theta \leq 35^\circ$.

3. Results and discussion

3.1. Structure of a gelatin layer

Fig. 2 illustrates WAXS photographs of a hardened gelatin layer with the primary beam perpendicular (a) and parallel (b) to layer surface. Three rings of uniform intensity (R_1 , R_2 and R_3) can be seen in the perpendicular incidence photograph. One pair of spots (E_0) and two pairs of arcs (E_1 and E_2) along the equator and three reflections (M_1 , M_2 and M_3), with a slightly meridional character, are observed in the parallel incidence photograph (Fig. 2a). The reflection E_0 , which appears at the border of the beam stop, has been confirmed using a longer sample–film distance. The vertical dark line in Fig. 2b, produced by sample absorption during recording, is used as a reference for macroscopic sample orientation. The calculated spacings, d , corresponding to the gelatin layer structure are collected in Table I. The identification of the diffracting planes present in the gelatin has been carried out by comparing the obtained spacings with those reported for collagen [9–12]. These values are also given in Table I. Values corresponding to R_1 , R_2 and R_3 rings in Fig. 2a coincide with those of M_1 , M_2 and M_3 (Fig. 2b), respectively. By analogy with

collagen, gelatin layers would contain individual chains (with interchain distances related to the broad R_2 and M_2 reflections) and triple-stranded helical rods (R_1 , M_1 , R_3 and M_3 reflections) entangled together. The intense equatorial reflection E_1 is associated to the lateral packing of triple-helical rods and the meridional lines M_1 and M_3 , are related to longitudinal spacings within the triple-helix. The arcing of these reflections indicate that most triple-helical rods are oriented parallel to the gelatin layer surface. Furthermore, since R_1 and R_3 are rings, triple-helix rods are randomly oriented with their axes parallel to the surface. Thus, from the two X-ray patterns of Fig. 2 it can be concluded that gelatin layers show planar orientation. Reflections E_2 , M_2 and R_2 , which are related to individual polypeptide chains, show that these chains should be arranged in a similar way to those of triple-helical rods. However, in this case, wider reflections suggest shorter coherent lengths. The shape of the E_0 spots ($d = 3.5$ nm) indicates the presence of well oriented structures of larger size, which could correspond to microfibrils formed by aggregation of triple-helical rods [13–15].

3.2. Dependence on preparation conditions

Although gelatin layers show planar orientation, we have used X-ray diffractometry to follow the structural changes that can take place in gelatin layers due to a selective variation of the preparation conditions. This technique enables us to obtain quantitative analyses faster than by means of the transmission camera. First it could be illustrative to compare the results obtained with the diffractometer and the transmission camera for a given sample (Fig. 3). A diffractogram measures the scattering intensity distribution along the equatorial line of the diffraction pattern, being enhanced by the intensity of those crystal reflections with diffraction planes parallel to the sample surface. The largest difference between spacing values obtained from both methods correspond to reflection E_0 (see Table I). This difference is probably due to variations in interfibrillar distances of triple-helix rods. Additional experiments also showed a random variation of the intensity of this diffraction line along the sample. For this reason the E_0 reflection was dismissed for any further analysis. The observed different spacings corresponding to reflections E_1 and E_2 are due to variations in layer hydration [6]. Packing of individual chains and triple-helical rods in the evacuated transmission camera increase and, consequently, structure spacings decrease. This fact was confirmed by dehydration of the sample in an oven at 120°C . Spacings obtained in the diffractometer after dehydration were 1.02 and 0.49 nm for reflections E_1 and E_2 , respectively—very close to those obtained in the transmission camera. On the other hand, the weak E_2 reflection did not show any variation due to preparation conditions of the gel (mean deviation of intensity $< 2\%$) and therefore we selected the E_1 reflection at 2θ ca. 8° as a reference. We found that the E_1 spacing, related to the lateral packing of triple-helix rods, is also

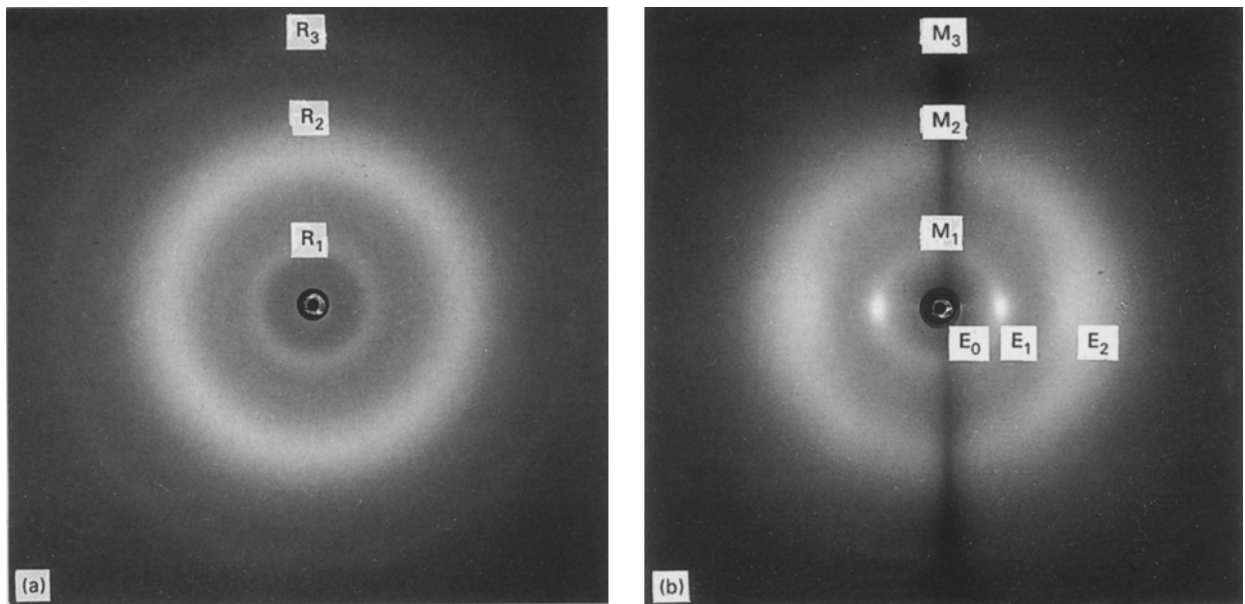


Figure 2 X-ray diffraction patterns of a hardened gelatin layer: (a) perpendicular incidence; (b) parallel incidence.

TABLE I Characteristic spacings of gelatin layer and collagen. Values in parenthesis have been obtained in the diffractometer

Reflection	Characteristic spacing (nm)	
	Gelatin layer	Collagen [9-12]
R_1, M_1	1.13	1.0
R_2, M_2	0.47	0.42
R_3, M_3	0.30	0.29
E_0	3.50 (2.54)	3.8
E_1	1.04 (1.14)	1.0-1.5 ^a
E_2	0.50 (0.54)	-

^aThe value depends on collagen hydration.

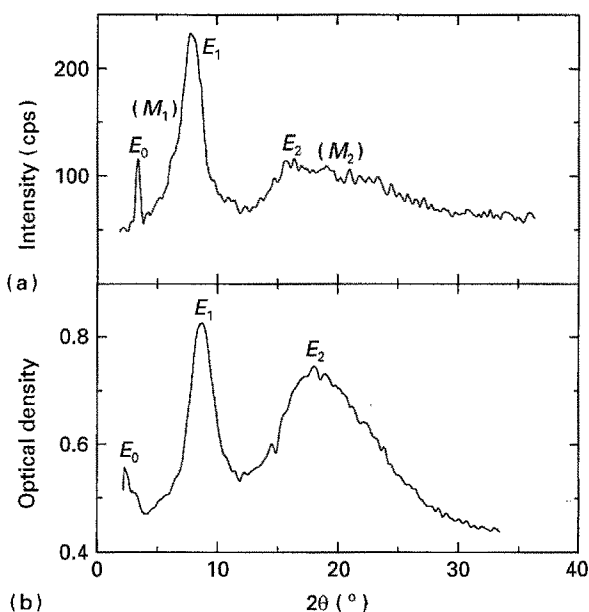


Figure 3 X-ray diagrams of a gelatin sample obtained by means of: (a) diffractometer; (b) transmission camera with parallel incidence arrangement and equatorial scanning.

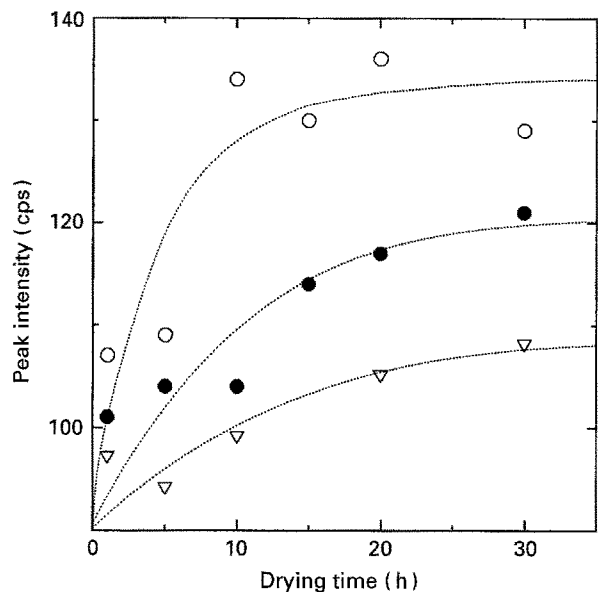


Figure 4 Peak height intensity of E_1 reflection as a function of drying time for different temperatures ($^{\circ}\text{C}$): \circ , 17; \bullet , 21; ∇ , 27.

independent of preparation conditions, but the peak height intensity strongly depends on temperature and drying time. Peak intensity, for a constant peak width, can be tentatively related to the amount of triple-helical rods in the crystallographic register. Fig. 4 shows peak height intensity as a function of drying time for various drying temperatures: 17, 21 and 27°C . The amount of crystalline triple-helical rods seems to increase with drying time in the first part of the experiment, remaining constant after ca. 15 h. It is further observed that triple-helix formation rate decreases with temperature. So the amount of triple-helical rods aggregated to crystalline regions in a 30×30 cm layer will be higher in the centre (longer drying time) than at the borders (shorter drying time).

Differences will be particularly noticeable in plates prepared by the conventional procedure described in the experimental section, where the drying temperature was 17 °C. In this case, drying time, in the centre and at the borders, varied by a factor of four from ca. 20 to ca. 5 h.

Experiments were also performed at two extreme values of temperature: 4 and 43 °C. At 4 °C, the peak height intensity of E_1 was slightly lower than that at 17 °C, probably due to the high viscosity of the gelatin solution when it was spread out onto such a cold glass support. On the other hand, the 43 °C drying temperature diffractograms did not present measurable diffraction peaks. This result agrees with the fact that gelation only takes place below 35 °C and, therefore, the amount of aggregated triple-helical rods is negligible [16].

A final question was to elucidate the role of the hardener agent. For this purpose a gelatin sample prepared at 17 °C, without chrome-alum, was examined by X-ray diffraction. We found, for both long and short drying times, crystalline reflections with the same spacings as those corresponding to hardened layers showing, however, a 25% higher peak intensity. In addition, the width at the half-height of the E_1 diffraction peak corresponding to unhardened layers was significantly smaller than that of hardened ones. It indicates that cross-linking by chrome-alum has a negative effect on the coherent packing of triple-helices, due to the bonding competition between the chrome-alum molecules and the single polypeptide chains. Taking into account that swelling results (Table II) show that layer hardening increases with increasing drying time, even for samples without chrome-alum, a possible route for obtaining hardened gelatin layers without hardener agent and enhanced formation of triple-helices and microfibrils can be envisaged.

3.3. Solar u.v. holoconcentrators

From the above discussion, and as a first step to overcome the lack of uniformity of large u.v. holoconcentrators, we propose the following method of preparation: chrome-alum hardened gelatin layers should be dried at 17 °C and a relative humidity of ca. 80% for 15 h, and then the relative humidity in the coating camera should be decreased to ca. 30%. In this way, drying at the corners can be slowed down to ca. 20 h, while the same process is completed at the centre after ca. 30 h, which means a time factor of only 1.5. Fig. 5 shows the variation of operating wavelength and bandwidth along the diagonal of a 30 × 30 cm concentrator recorded in a hardened gelatin layer which was prepared according to the new method. For the sake of comparison, data corresponding to a concentrator made through the conventional procedure are also included. We can see that uniformity is appreciably ameliorated after these simple changes, which shows the influence of gelatin structure in the spectral response of holographic mirrors. These results also explain the better uniformity of winter holograms compared to summer holograms when no special care is

TABLE II Degree of swell of gelatin layers for short and long drying times

Chrome-alum (%)	Swelling (%)	
	Short drying time (ca. 5 h)	Long drying time (ca. 20 h)
0	1150	800
2	550	370

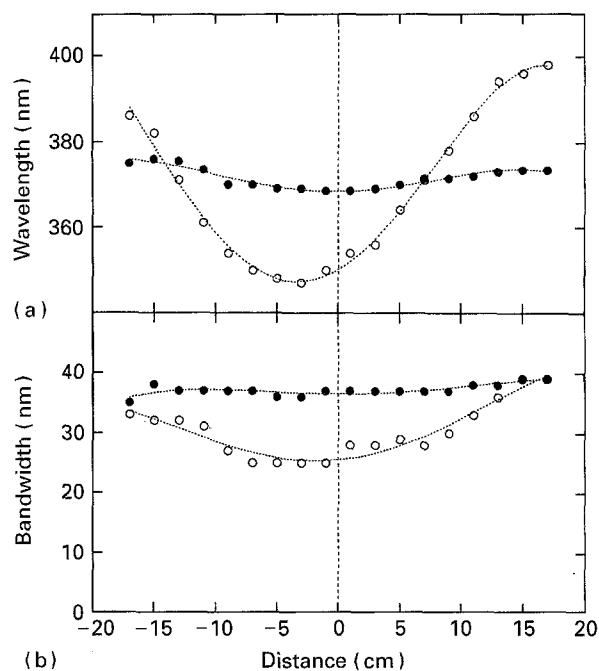


Figure 5 Operating wavelength (a) and bandwidth (b) variations along the diagonal of 30 × 30 cm u.v. holoconcentrators recorded in a hardened gelatin layer using the proposed preparation method (●) and the conventional method (○).

taken. In summer the laboratory air conditioning significantly decreases the relative humidity inside the coating camera which leads to a faster drying of gelatin layers.

4. Conclusions

X-ray diffraction patterns show that hardened gelatin layers are basically formed by individual gelatin chains partly associated as triple-stranded helical rods preferentially parallel to the gelatin surface. These rods are, in turn, partly associated in microfibrils as in native collagen. However, scarcity and width of reflections indicate a low degree of order. Microfibrils, as well as triple-helices, are related to the strength of the gelatin layer and further studies should be done in order to adjust its optimum degree of hardening. We note that reducing the amount, or even suppressing the hardener agent, would increase stability, sensitivity and reproducibility of dichromated gelatin layers for holography.

Diffraction studies show that layer structure is affected by gelatin layer preparation conditions. The

hardener agent has a negative effect on triple-helix formation and leads to an unhomogeneous packing. The amount of crystalline triple-helices also depends on gelation temperature and drying time: it increases with decreasing temperature, reaching a maximum value for each temperature at long drying times.

Gelatin hardening, which depends on triple-helix formation and chrome-alum cross-linking, increases with layer drying time. Since layer drying takes place from edges toward the centre, as if it were a big drop of water, a shorter drying time leads to the centre-edge lack of uniformity which has been frequently found in large sized holograms. We have shown that a simple method for improving uniformity of large holoconcentrators consists in maintaining a relative humidity > 80% in the coating camera. As a consequence, layer drying is not completed before 20 h.

Future research will be concerned with structure evolution throughout different stages of dichromated gelatin processing. It should clarify the poorly understood mechanism of hologram formation and be a valuable help for optimizing specific processes such as the one followed to obtain u.v. solar holoconcentrators.

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