

Distribution Feature of Calcium with Respect to Molecular Weight in Calcium-Containing Photographic Gelatin

Huang Bixia, Xu Loo, Yin Yimei, Sue Jun, Zhang Weixiang
Dept. of Applied Chemistry, University of Sci. & Tech. of China, Hefei, Anhui, China

Abstract

Calcium has the important influences both on photographic performances of emulsion and on physico-mechanical properties of gelatin. In this paper, the chemical form of calcium added to gelatin is studied, which demonstrates a similar situation to self-calcium of gelatin.

Introduction

It is made clear that calcium affects chemical ripening and physical ripening processes of silver halide photographic emulsions[1,2] as well as significantly restrains the growth of silver halide microcrystals[3]. Recently, it is found,[4,5] that Ca^{2+} ions doped in the silver halide emulsion not only retard the process of chemical sensitization and inhibit spectral sensitization at long wavelength, but also cause a shift of the dielectric absorption peaks of the AgX emulsion towards lower frequencies. The study on physico-mechanical properties of gelatin showed[6] that, at gelatin concentrations between 2% and 4%, the adhesion ability of a gelatin solution on a dry or wet gelatin(emulsion) layer depends on the Ca^{2+} content of the coating solution. It thus is necessary to have at least 100ug Ca per gram of gelatin to guarantee a perfect adhesion and a perfect coating. Calcium strongly decreases the electric potential carried by gelatin and impairs the colloidal stability of gelatinous dispersions in the PH range of 6 to 8.[7] The efficiency of the macromolecule polyanionic thickeners is suppressed dramatically in the presence of foreign calcium and more thickener is needed in order to obtain the same viscosity of gelatin solution as one of gelatin solution without addition of Calcium. In addition, Calcium influences the bloom of gelatin solution and damages the degree of order structure in gelatin.[6,8]

These previous studies were made by means of so-called foreign calcium doping method, i.e. some amounts of calcium salt were added into gelatin solutions or gelatin-silver halide emulsions. It is well known that among main raw materials utilized in photographic emulsion making the amount of photographic gelatin is more than others such as silver nitrate, halide and coupler. In general, calcium content in the bone inert gelatin used is about thousands of microgram per gram of gelatin, as shown in Table 1, which is much more than one in others. It means that for the effects of calcium on physico-mechanical and photographic properties of emulsion coating calcium in gelatin is in the dominent position. Therefore, it is reasonable to ask if the results from calcium doping tests above can represent the actural behavior of calcium in gelatin. It is a better way for answering to explore chemical form of calcium, both self-calcium and foreign calcium added in gelatin, because chemical forms of metal ions in gelatin may affect their activities and may thus cause different photographic effects.

In general, the raw materials of high quality gelatin are hard cattle bones of animal. There are however, many kinds of Calcium in the bones. Every variety of calcium may cause different biochemistry effects. According to our earlier study,[9] self-calcium of gelatin is bound to the macro-molecules and no free calcium is found. There has, however, not been any report to deal with chemical form of foreign calcium added in with chemical form of foreign calcium added in gelatin so far. There appear the problems whether the foreign calcium doped in gelatin and the self-calcium of gelatin exists as same chemical forms and thus have same effects on the gelatin solution or emulsion. In this paper, the chemical form of the calcium added in gelatin is investigated.

Experimental

Materials and Methods

The test gelatins are listed in Table 1.

The gel filtration is used for separation of the gelatin components in respect to molecular weight. The separation column with sepharose 4B gel(Pharmacia. Sweden) has following specifications: bed height, 800 mm; internal diameter, 13mm. $0.2molL^{-1}$ sodium chloride solution is used as eluate and it's flow rate is 12ml/h.

The protein content of every fractional solution is monitored with a spectrophotometer at 230 nm. Calcium is determined using the flame Atomic Absorption Spectrometer.

Then neighbor fractions are mixed and concentrated to about 5ml for determination of phosphorus content. After removing of the isolated NaCl salt, the concentrated solutions are digested with the mixed reagents of 1ml perchloric acid and 5ml nitric acid. The residues are dissolved by 10ml pure water. Then 5ml of a mixed reagent with 2.5ml of $3moll.^{-1}$ sulfuric acid solution, 1.0ml of 2.5% ammonium molybdate solution, 0.5ml of pure water and 1.0ml of 10% ascorbic acid solution is added to the water solution of residue. Mix and stand the solution at 45°C for 5 minutes. The blue complex appears during this period. Cool the solution with the blue complex, which is stable at the room temperature, and then measure the absorbances at 823nm in 2-cm cells against a blank.

Table 1. The main characteristics of the test gelatins

Gelatin	Ca content (ug/g)	P content (ug/g)	bloom (6.67% Soln.)	Viscosity (cp) (6.67% Soln, (60°C)	PH 2% Soln. 37°C
CH	1971.4	22.6	298	6.74	5.7
FD	4256.6	15.4	235	6.18	5.6

Experimental Procedure

1. Separation and Analysis of the Gelatin Solution 1.0ml of 0.5% gelatin solution is applied to the column and separated. 50 fractions are collected and used for determinations of protein. Calcium and phosphorus.
2. Separation and Analysis of the Gelatin Solution with Foreign Calcium — Prepare 0.5% gelatin solution with addition of 3000ug foreign Calcium per gram of gelatin and stand it for 60min at 60°C in order to approach the process of chemical ripening or emusion. Then the experimental procedure 1 is repeated using this solution without determination of phosphorus.
3. Separation and Analysis of the Gelatin Solution with Foreign Phosphorus—Prepare 0.5% gelatin solution with addition of 100ug foreign phosphorus per gram of gelatin and stand it for 60min at 60°C in order to approach the process of chemical ripening or emulsion. Then the experimental procedure 1 is circulated with this solution.

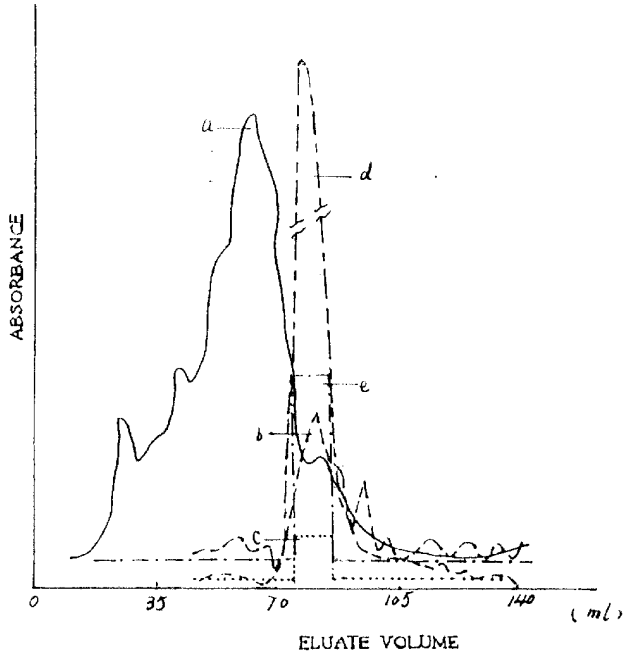

Figure 1.

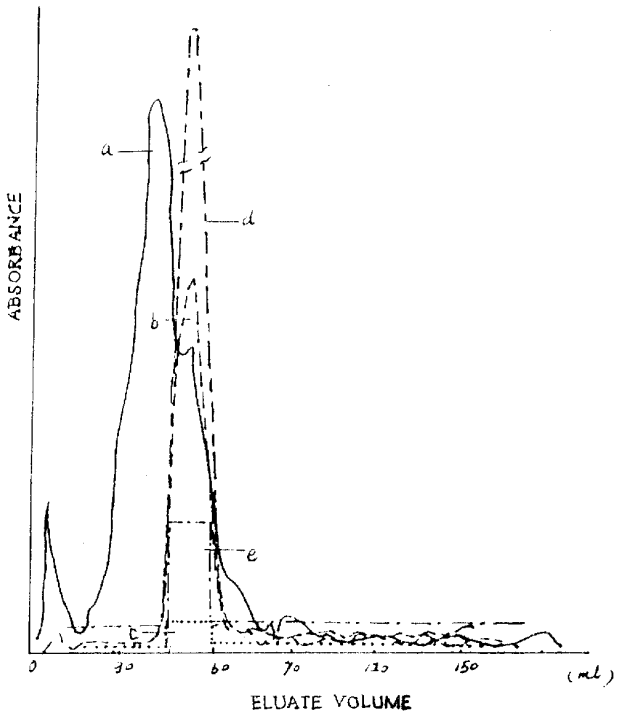

Figure 2.

Results

The distribution curves of protein, calcium and phosphorus with respect to molecular weight for various gelatin samples are shown in Figure l and Figure 2. respectively. In the figures the curve a represents the distribution of gelatin protein; the curve b-the distribution of self-calcium of gelatin; the curve c-the distribution of self-phosphorus of gelatin; the curve d--the distribution of calcium in gelatin with foreign calcium; the curve e--the distribution of phosphorus in gelatin with foreign phosphorus. The horizontal scales are roughly a logarithmic scale of molecular weight. The vertical scales show the absorbance of protein, calcium and phosphorus complex respectively, which means their related contents.

Discussion and Conclusion

The basis of the high quality gelatin preferably consists of pure, degreased and hard cattle bones. The great majority of calcium in the bone exists as hydroxyapatite and disperses in collagen net by means of the phosphoprotein. The phosphoprotein, one of the major constituents of the noncollagenous proteins found in all vertebrate calcified tissues, have been postulated to play an important role in the initiation and regulation of calcification[10,11]. In general, the first step in the preparation of gelatin is an acid treatment of the ground and calibrated bones in order to eliminate the calcium and magnesium phosphates. It is followed by an alkaline programmed hydrolysis of the residual collagen with calcium hydroxide. During this process, the

polypeptide again takes up calcium ions up to saturation. The following purification procedure makes the calcium controlled at the lower level.

Comparing the calcium distribution curve-b with the protein distribution curve-a in Figure l and Figure 2 respectively, we verify that the calcium in FD and CH gelatins are all the organic calcium and there is no free calcium. It is observed from the curve-c that the fractions with high concentration of calcium contain the phosphorus component and the majorities of self-phosphorus of gelatin is also at these areas. It implies that the calcium ligands may be the phosphorylated proteins. In the curve-d, which means the distribution of calcium in gelatin with added calcium, the peaks of these curves appear at the same positions as ones in the curves-b. It proves that the foreign calcium is bound to same kind of ligand yet as the self-calcium of gelatin does. Even the amount of foreign calcium doped in gelatin is much greatly increased to 200 mg per gram of gelatin, the same situation also occurs. It is found by use of comparition of curve-e and curve-b that the foreign phosphorus is also combined to the same species as the self-calcium of gelatin does. These facts above show the characters of calcium ligands in gelatin, i.e. theses ligands are phosphorylated protein and have a strong affinity for both calcium and phosphonic groups. As it happens, these are the natures of the phosphoproteins.[12,13] Consequently, we suggest that calcium added in gelatin and self-calcium of gelatin have same of similar chemical forms because they are both bound to same phosphoprotein.

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