

# This Week's Citation Classic®

**Tiselius A, Hjertén S & Levin Ö.** Protein chromatography on calcium phosphate columns. *Arch. Biochem. Biophys.* 65:132-55, 1956.  
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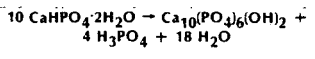
A method for the preparation of a stable form of calcium phosphate (hydroxyapatite) is described. Elution of the proteins to be separated is achieved by an increase in the concentration of the eluent (phosphate buffer). Low-molecular-weight material shows little or no adsorption to hydroxyapatite. [The SCI® indicates that this paper has been cited in over 1,170 publications since 1956.]

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September 20, 1985

In 1954 Östen Levin, a friend of mine and one of the authors of the above paper, began with me our studies toward a Swedish *fil. lic.* exam at the Institute of Biochemistry, Uppsala University. Our tutor was Arne Tiselius, who at that time was developing calcium phosphate,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  (brushite), as an adsorbent for protein chromatography<sup>1</sup> (in 1948 he received the Nobel Prize for his fundamental work on electrophoresis and chromatography).

One of the first research tasks Tiselius gave me was to find out how the adsorption of proteins to brushite was affected by temperature. For that purpose a glass tube packed with brushite was immersed in a water bath of 40° C. The column was washed with 0.005 M sodium phosphate, pH 6.9, and the approach to equilibrium was monitored by pH measurements. To my surprise the pH of the effluent was around 5 and remained there for days. I also observed that the appearance of the adsorbent at the top of the column was slightly different from that in the rest of the column. Tiselius asked a colleague to make an X-ray analysis of the top layer, which appeared to consist of hydroxyapatite. The brushite had thus been converted to hydroxyapatite according to the following formula:<sup>1</sup>



The transformation of brushite to hydroxyapatite also occurs below 40° C, although at a lower rate. Therefore, we decided to use the stable hydroxyapatite as adsorbent instead of the unstable brushite introduced previously by Tiselius.<sup>1</sup> The above experiment and formula indicated that brushite can easily be converted to hydroxyapatite at elevated temperatures upon removal of the phosphoric acid formed. The method for the preparation of hydroxyapatite described in the cited paper thus involved boiling of a suspension of brushite at a high pH.

Most methods for the separation of biopolymers (such as proteins and nucleic acids) are based on differences in their charge or size. In hydroxyapatite chromatography, however, other parameters are of more importance, particularly the number of available carboxylic and phosphoric groups in the biopolymers to be separated.<sup>2,3</sup> This unique separation mechanism often gives separations that are not possible to achieve with more conventional methods, such as molecular-sieve chromatography (gel filtration) and ion-exchange chromatography. I believe that this is the main reason hydroxyapatite has been so popular for the purification of both proteins and nucleic acids. Another reason may be that hydroxyapatite differs from ion exchangers in the respect that ions other than phosphate ions have little influence on the adsorption of proteins.<sup>4,5</sup> The adsorption is even strong in the presence of sodium dodecyl sulfate and high concentrations of sodium chloride, which may be of importance in the design of an experiment.<sup>4,6</sup> I am surprised that the property of this adsorbent to permit the separation of low-molecular-weight substances from macromolecules, is not well known, although this can be utilized, for instance, for the removal of proteins prior to high-pressure liquid chromatography (HPLC) analyses of drugs in human serum.

The paper's title refers to the separation of proteins. Hydroxyapatite is, however, also a useful adsorbent for the fractionation of nuclei acids.<sup>2</sup>

One can expect hydroxyapatite to be widely used also in the future for the separation of biopolymers, particularly since it has recently become commercially available as an HPLC adsorbent.

1. Tiselius A. Chromatography of proteins on calcium phosphate columns. *Ark. Kemi* 7:443-9, 1954. (Cited 70 times since 1955.)
2. Bernardi G. Chromatography of nucleic acids on hydroxyapatite. *Nature* 206:779-83, 1965. (Cited 205 times.)
3. Bernardi G & Kawasaki T. Chromatography of polypeptides and proteins on hydroxyapatite columns. *Biochim. Biophys. Acta* 160:301-10, 1968. (Cited 105 times.)
4. Hjertén S. Calcium phosphate chromatography of normal human serum and of electrophoretically isolated serum proteins. *Biochim. Biophys. Acta* 31:216-35, 1959. (Cited 110 times.)
5. Levin Ö. Column chromatography of proteins: calcium phosphate. *Meth. Enzymology* 5:27-32, 1962. (Cited 305 times.)
6. Moss B & Rosenblum E. Hydroxyapatite chromatography of protein-sodium dodecyl sulfate complexes. *J. Biol. Chem.* 247:5194-8, 1972. (Cited 140 times.)