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Note

Purification of potassium phosphate for high-performance liquid chromatography

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Potassium phosphate buffers, while having several advantages over other solvent systems for many high-performance liquid chromatography (HPLC) applications, suffer from a serious disadvantage, namely a high background absorption in the UV region. This is true for reagent-grade phosphates of every supplier tested. The problem becomes particularly acute when very small amounts of substances requiring high concentrations of buffer for elution are to be separated, a typical example being the separation of nucleoside triphosphates on ion-exchange columns. Although this absorption is obviously due to an impurity, several methods of purification fail to correct the problem. The only published method[1] for this purpose involves a Dowex 1-X8 purification and a series of time- and material-consuming recrystallizations.

We have been successful in cleaning up our phosphate buffers by the use of Chelex-100, a Bio-Rad (Richmond, CA, U.S.A.) chelating carboxylic acid cation exchanger. Chelex-100 is a styrene–divinylbenzene copolymer containing paired iminodiacetate ions which chelates preferentially transition metals, even in highly concentrated salt solutions. We are routinely passing an 1 *M* solution of KH_2PO_4 through a column of Chelex-100 (100–200 or 200–400 mesh) at a rate of about 1 ml/min · cm^2 of bed. This treatment effectively removes most of the UV absorbance from the phosphate, to the extent that a gradient reaching 1 *M* concentration can be used with full scale absorbance at 254 nm of 0.08 or 0.04 with a baseline shift of less than 5% or 10%, respectively.

The Chelex-100 is suspended directly in 1 *M* KH_2PO_4 and the slurry used to pack the column. The eluent is collected after 100 ml have passed through. Over 20 liters of 1 *M* solution can be purified through a 120-ml (250 × 25 mm) column without regeneration of the resin. Thus, the process is very economical even if the resin is discarded after use, but regeneration is possible following the manufacturer's instructions.

Fig. 1 represents a comparison of the UV spectra of the 1 *M* KH_2PO_4 solution before and after Chelex-100 purification. The nature of the material removed by the treatment has not been investigated. It is, however, of interest to note that the impurity accumulates on anion-exchange columns at concentrations lower than 1 *M*. Thus, if several runs are performed with gradients reaching up to 0.60 or 0.70 *M*, a subsequent 1 *M* elution will slowly remove large amounts of UV absorbing material. For the same reason, even if a concentration of 1 *M* is not routinely reached

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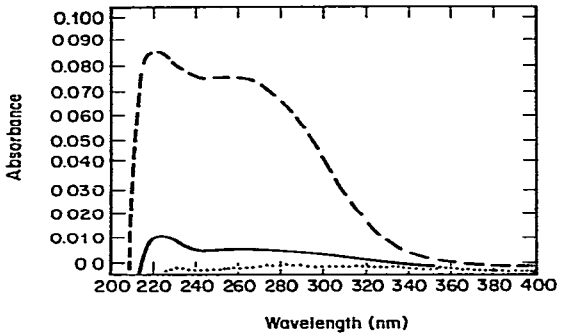

Fig. 1. UV spectra of 1 *M* KH_2PO_4 before and after purification with Chelex-100. Both unpurified (broken line) and purified (solid line) solutions were read against distilled water which had been passed through a Chelex-100 column. Distilled water not subjected to Chelex-100 treatment gave the spectrum indicated by the dotted line.

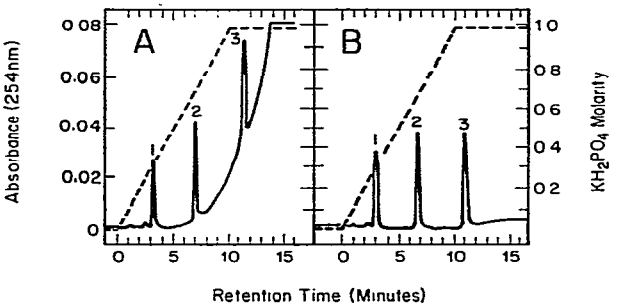

Fig. 2. Comparison of baseline quality before and after purification of KH_2PO_4 by Chelex-100. Instrument: Varian 5020; column: Varian Micropak AX-10, 30 cm × 4 mm I.D.; flow-rate, 3 ml/min; program: from 10 m*M* to 1 *M* KH_2PO_4 in 10 min. Peaks: 1 = AMP (0.3 nmoles); 2 = ADP (0.4 nmoles); 3 = ATP (0.6 nmoles). Full scale absorbance, 0.08. A, unpurified KH_2PO_4; B, purified KH_2PO_4.

in a particular separation, the quality of the baseline progressively deteriorates when unpurified buffers are used. A prolonged purge with purified buffer will eventually restore a stable low baseline.

The dramatic effect of Chelex-100 purification on baseline quality is illustrated in Fig. 2, which compares the separation of nanomole quantities of AMP, ADP and ATP on an AX-10 (Varian, Palo Alto, CA, U.S.A.) anion-exchange column with a 0.01 to 1 *M* gradient of KH_2PO_4, before and after treatment. The baseline goes off-scale with the unpurified buffer (Fig. 2A), while it increases by only 4% with the Chelex-purified buffer (Fig. 2B). Thus, picomole amounts of triphosphates can be accurately determined, without elaborate and uncertain baseline corrections, using Chelex-purified buffers.

REFERENCE

1 H. W. Shmukler, *J. Chromatogr. Sci.*, 8 (1970) 581.