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METHOD FOR THE SYNTHESIS OF 1-HYDROXY-ETHYLIDENE-1, 1-DISODIUM PHOSPHONATE (HEDSPA): A SKELETAL SEEKING RADIOPHARMACEUTICAL AFTER LABELING WITH ^{99m}Tc

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A method for the synthesis of the skeletal seeking agent, 1-hydroxy-ethylidene-1, 1-disodium phosphonate (HEDSPA), is presented. The method can be performed in any laboratory equipped with a suitable hood by combining phosphorous trichloride (PCl_3) and acetic acid (CH_3COOH). The diphosphonate remains chemically stable in crystalline form or in solution.

The skeletal system has recently been investigated with ^{99m}Tc-labeled radiopharmaceuticals (*1–6*). Inorganic polyphosphate has produced excellent bone scans when administered as ^{99m}Tc(S_m)-polyphosphate (*1*). However, this material remains chemically stable for only a few months, losing one or two phosphate units per month due to depolymerization (*1*). We chose to investigate a more chemically stable group of "bone-seeking" compounds; the organic diphosphonates. On the basis of various experimental studies (*7–10*) showing the diphosphonates preventing the deposition of calcium phosphate and several clinical studies showing these compounds favorably affecting the progress of certain bone disorders (*11–13*), we began an investigation to determine whether a suitable ^{99m}Tc-diphosphonate bone-imaging radiopharmaceutical could be developed.

The diphosphonate we chose to investigate is 1-hydroxy-ethylidene-1, 1-diphosphonic acid (HEDPHA). Its disodium salt (HEDSPA) tags easily with stannous-reduced $^{99m}TcO_4^-$ (*4–6*). Our Division of Nuclear Medicine has performed over 1,500 bone scans without adverse effects (*14–19*).

This paper outlines a method for the synthesis of the skeletal seeking agent 1-hydroxy-ethylidene-1, 1-disodium phosphonate (HEDSPA).

MATERIALS AND METHODS

A. Synthesis of 1-hydroxy-ethylidene-1, 1-diphosphonic acid (HEDPHA), a method from that of Albright and Wilson (*18,19*).

1. Chemicals
   a. Glacial acetic acid (reagent grade).
   b. Phosphorus trichloride (reagent grade). Note: Both chemicals are poisonous and can cause severe burns when in contact with skin and mucous membranes.
2. Method: The HEDPHA is prepared in a well-ventilated hood as follows:
   a. Into a 500-ml triple-necked round-bottom flask provided with a thermometer and a magnetic stirring bar, place 180 gm of glacial acetic acid.
   b. Slowly pour 68.8 gm of phosphorus trichloride into the flask and stir gently for 10 min at room temperature.
   c. The reaction flask is then placed in an ice-water bath and gently mixed for 30 min at 5°C.
   d. The flask is removed from the ice-water bath and a dry distillation condenser is attached. The apparatus is then placed in an oil bath and the reaction mixture heated slowly to 60°C over a 2-hr period.
   e. The mixture is heated to 120–130°C for 20–30 min. At this temperature the mixture is then steam distilled until there is a slight trace of acid (pH 5) in the distillate.
   f. The semisolid acid product (HEDPHA)

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is cooled to room temperature and stored under refrigeration.

B. Qualitative analysis of HEDPHA-titration with NaOH.
   1. Chemicals
      a. Sodium hydroxide pellets, ACS grade.
      b. Distilled water for injection.
   2. Method
      a. One ml of HEDPHA is transferred to a beaker containing 10 ml of distilled water and a magnetic stirring bar. The solution is mixed until dissolution of the HEDPHA is complete.
      b. A 50-ml buret is filled with a standard 1 *N* solution of NaOH freshly prepared for titration.
      c. A pH electrode is placed in the diluted HEDPHA solution and the NaOH is then added slowly in 0.1-ml increments. The change in pH is noted after each addition of NaOH.
      d. A linear plot of the titration curve is then constructed as illustrated in Fig. 1.

C. Elemental analysis HEDPHA: One ml of the HEDPHA product is removed from the stock solution for elemental analysis to determine the percentage of carbon, oxygen, phosphorus, and hydrogen (Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.).

D. Quantitative analysis of HEDPHA: The quantity of HEDPHA present per milliliter of product can be determined from the titration data obtained previously by following simple stoichiometric relationships as outlined in the literature (*20,21*).

E. Preparation of 1-hydroxy-ethylidene-1, 1-disodium phosphonate (HEDSPA) stock solution.
   1. Method
      a. 100 ml of sterile-distilled water is transferred to the remaining HEDPHA, and the solution is mixed until dissolution is complete.
      b. The solution is then titrated with 1 *N* NaOH to pH 9.0.
      c. The number of grams of HEDSPA present in the solution is then calculated from simple stoichiometric relationships (*22, 23*).
      d. With the aid of a rotating vacuum evaporator the HEDSPA solution is brought to dryness. The resulting solid is dissolved in a minimum of hot water to produce a supersaturated solution. Approximately ½ ml of hot ethanol is added to this mixture, and the resulting solution is immediately cooled in an ice bath where crystallization occurs. The crystals are then dried in a rotating vacuum evaporator.
      e. The following quality control procedures were performed on the crystallized product:
         (1) Elemental content; C,H,O,P.
         (2) Nuclear magnetic resonance (NMR).

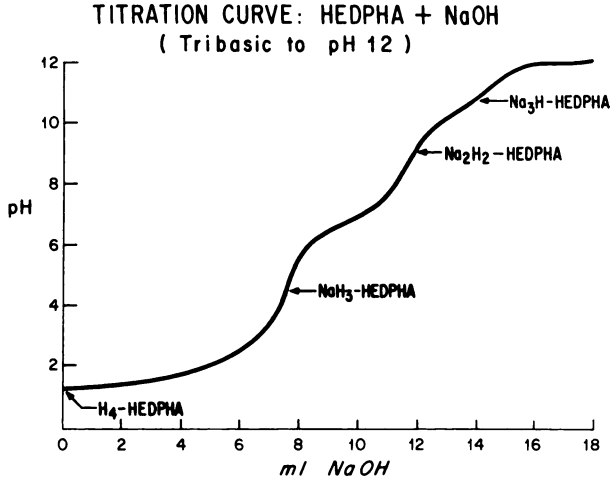

FIG. 1. Tribasic titration curve for HEDPHA showing equivalence points at pH values 4.4, 8.5, and 10.7.

The ^{99m}Tc-labeling procedure and the quality control of the ^{99m}Tc-HEDSPA (*4–6*) human studies (*14–19*) and comments concerning its long term (*24*) and acute (*25*) toxicity are reported elsewhere.

RESULTS AND DISCUSSION

In the synthetic procedure for HEDPHA, the handling of acetic acid and phosphorus trichloride necessitates the use of a well-ventilated hood. Hydrochloric acid vapor and acetic acid vapor are produced during the procedure and great care should be taken to limit exposure to them. After 15 min of heating at 120–130°, a semisolid gel is formed. The steam distillation step which follows removes acetic acid as a vapor and, upon cooling, a viscous liquid results.

The qualitative analysis for HEDPHA is based on simple titration of an organic acid. The titration

TABLE 1. ELEMENTAL ANALYSIS

Molecule	%C	%H	%O	%P
Calculated				
HEDPHA	7.77	5.28	62.99	22.41
HEDSPA	7.76	2.90	52.03	19.65
Theoretical				
HEDPHA-$(HOH)_4$	8.64	5.80	63.29	22.27
HEDSPA-$(HOH)_4$	7.40	3.40	54.00	19.70

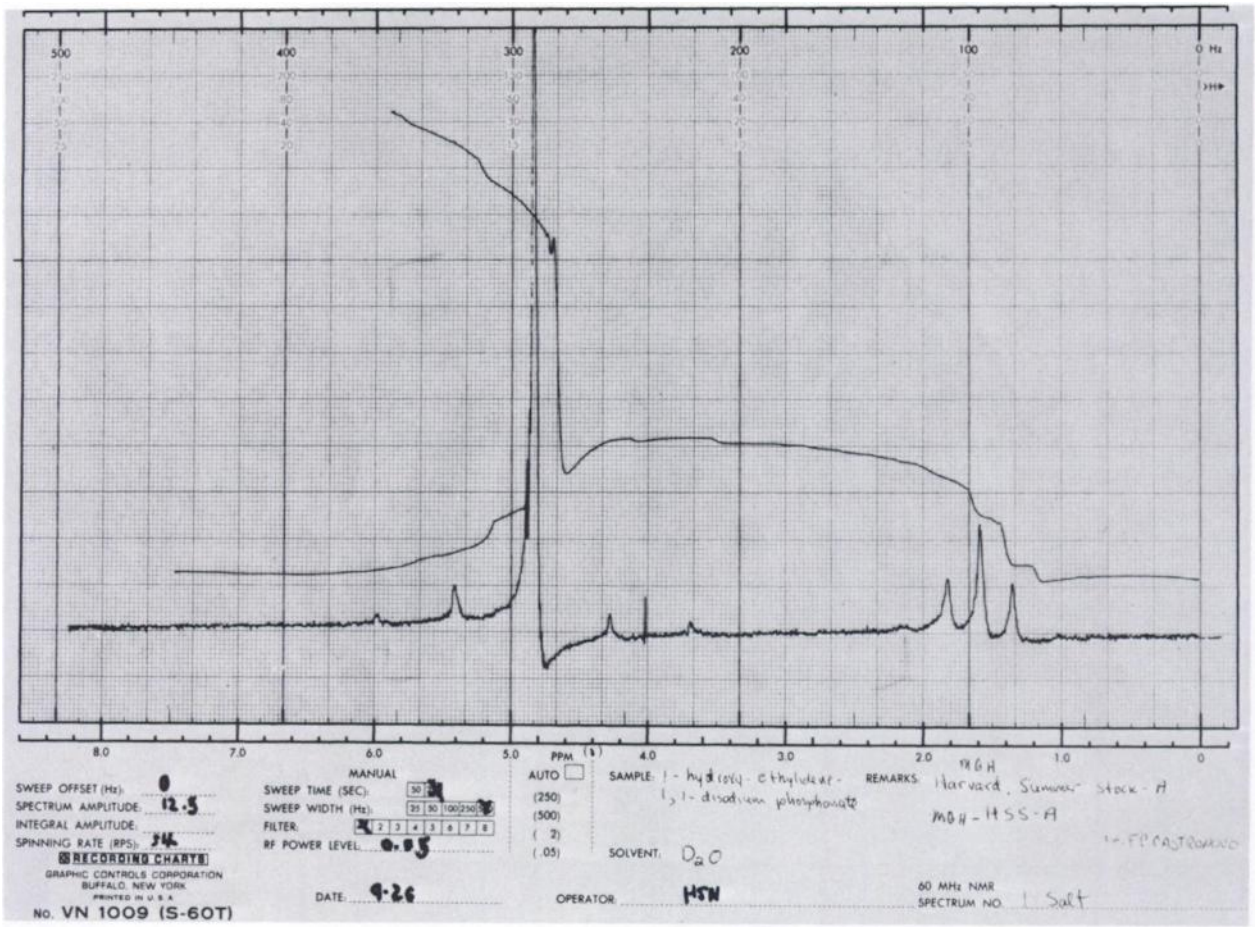

FIG. 2. Nuclear magnetic resonance spectrum (NMR) of HEDSPA illustrating "triplet" curve representative of phosphorus splitting of methyl hydrogens.

of this acid with a standardized solution of sodium hydroxide resulted in three isoelectric points at the following pH values: 4.4, 8.5, and 10.7 (Fig. 1). The first two points occurring close to those values for phosphoric acid; pH's, 4.0 and 9.0 (*22*). The results from the elemental analysis of the HEDPHA are shown in Table 1.

The preparation of the disodium salt (HEDSPA) was accomplished by titrating the HEDPHA product with sodium hydroxide. The HEDSPA was then vacuum evaporated to dryness, redissolved, and then crystallized. This product was subjected to an elemental analysis similar to that of the HEDPHA. The results of this study are also shown on Table 1. The nuclear magnetic resonance results are illustrated in Fig. 2. This study was performed to further characterize the product as illustrated by the "triplet" of peaks representing the phosphorus splitting of the methyl hydrogens. This paper outlines a method for the synthesis of the skeletal seeking agent 1-hydroxyethylidene-1, 1-disodium phosphonate.

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