

## Determination of the Occluded Calcium Hydroxyapatite in Polystyrene Beads

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### INTRODUCTION

The bead polymerization of styrene in the presence of insoluble suspending agents and various initiators has been described by several authors.<sup>1-3</sup> The most widely used insoluble salts are perhaps the calcium hydroxyapatites (CHAP), deflocculated with anionic surface active agents.<sup>4-6</sup> These stabilizers are very effective at high monomer-to-water ratios and produce polymers of exceptional color and clarity.

Although most of the apatite can be removed by a mild acid washing, some of it, probably imbedded in the beads, remains in the polymer. The purpose of this work was to determine the concentration of occluded CHAP in polystyrene beads and to correlate this concentration with the bead size. Since the gravimetric determination of small quantities of CHAP in the polymer proved to be quite inaccurate, owing to losses during calcination, the "tracer method" was used. Styrene was polymerized in the presence of CHAP precipitated from calcium acetate and disodium phosphate containing known amounts of disodium phosphate labeled with P<sup>32</sup> of high specific activity. Under these conditions, the concentration of CHAP in the polymer was proportional to the number of disintegrations per second.

### EXPERIMENTAL

#### 1. Materials Used

Commercial styrene was purified by steam distillation. Calcium acetate monohydrate and disodium phosphate dodecahydrate were "Baker Analyzed" reagents. The benzoyl peroxide was obtained from the Lucidol Company. A radioactive solution of disodium phosphate labeled with P<sup>32</sup>, obtained from Oak Ridge National Laboratories, was used for the preparation of the labeled calcium hydroxyapatite.

#### 2. Preparation of the Radioactive Beads

Into a 4-gallon reactor fitted with an impeller type stirrer, nitrogen inlet, reflux condenser, and a thermocouple, were added 7 l. of distilled water and

400 ml. of an aqueous solution containing 33.4 g. of calcium acetate monohydrate. To this solution heated at 90°C. were added simultaneously with stirring, 50 ml. of radioactive solution (containing Na<sub>2</sub>HPO<sub>4</sub> labeled with P<sup>32</sup>) and 500 ml. of an aqueous solution containing 38.5 g. of disodium phosphate monohydrate, 5.76 g. of NaOH, and 0.15 g. of Nacconol NRSF, sodium alkylaryl sulfonate. After 15 min. at 90°C., 4200 g. of styrene containing 7.68 g. of benzoyl peroxide was added. Polymerization was carried out with stirring in a nitrogen atmosphere at 100°C. for 24 hr. The beads were steam stripped, washed with water, and dried. The conversion of the monomer was 96.5%.

#### 3. Counting of Polystyrene Chips

Ten grams of radioactive beads was pressed into 1/2 × 1 × 1-inch chips in a four-cavity, chromium-plated mold and counted in a conventional type microwindow Geiger counter. To convert the number of counts per minute (c./min.) to concentration of CHAP, it was necessary to determine the c./min. of polystyrene chips containing a known amount of radioactive solution. These standards were obtained by slurring 10-g. samples of non-radioactive beads with a known amount of active solution in the cavities of the mold. After drying at 80°C., the beads were pressed at 150°C., and the resulting chips counted. The counts of the opposite surfaces were close enough to be averaged. All counts were corrected to a standard date.

### DATA AND DISCUSSION

Preliminary experiments, conducted in 8-oz. polymerization bottles with 0.2 g. of labeled CHAP and 50 g. of monomer, showed qualitatively that the residual concentration of calcium hydroxyapatite is a function of the size of the beads. The larger the beads, the larger the concentration. To investigate this relationship further, a large batch of beads was prepared in a 5-gallon reactor as described in the experimental section, and fractionated into eleven fractions by screening. About 80% of the beads had a diameter between 0.4 and

TABLE I. Concentration<sup>a</sup> of CHAP in the Beads

Fraction no.	Average diameter, mm.	Total CHAP, p.p.m.	Ocluded CHAP, p.p.m.	Surface CHAP, p.p.m.
1	>1.40	720	460	260
3	0.92	375	140	235
4	0.78	487	73	414
5	0.66	3,300	36	3,264
7	0.38	6,100	26	6,074
11	<0.20	20,740	4,540	16,200

<sup>a</sup> All concentrations are expressed in p.p.m. of polymer.

0.7 mm. Unwashed beads from six of the eleven fractions were pressed into chips and counted. The results reported in Table I, column 3, show the total concentration of CHAP in the polymer, representing both the apatite which was imbedded in the surface and occluded inside the beads. The concentration of occluded CHAP, column 4, was obtained by counting a portion of the six fractions, which had been washed twice with dilute hydrochloric acid at pH 2. The concentration of CHAP on the surface of the beads (column 5) was obtained by difference.

The data of column 5 show, at least qualitatively, that the *smaller beads* contain a larger concentration of apatite on their surface, than the *larger ones*. This is to be expected since this concentration should be proportional to the surface area per unit weight. The data, however, are not quantitative because they were derived from measurements made on the unwashed beads. It is possible that during the screening, portions of the loose apatite might have collected in the fractions containing the smaller beads, thus vitiating the results. On the other hand, the determinations made on the washed beads are meaningful. The data of column 4 show that the large beads (discounting the polymer dust of fraction 11) contain more occluded apatite per unit weight than the small ones. These results suggest that the larger beads are formed during the "sticky stage" by coalescence of smaller ones carrying apatite imbedded in their surface.

It is interesting to note that the very fine polymer of fraction 11 contains more residual CHAP than any other fraction. This polymer is rather unstable to heat and discolors appreciably on molding at 150°C. The formation of polymer dust has been attributed to grinding of the surface of the beads caused by stirring.

The data of Table II show that two washings with dilute hydrochloric acid at pH 2 are sufficient to dissolve the apatite imbedded in the surface of the beads.

TABLE II. Influence of the Acid Washing on the Residual Concentration of CHAP

Fraction no.	Average diameter, mm.	Number of washings at pH 2	CHAP, p.p.m.
5	0.66	2	34
		3	30
		4	35

The average concentration of residual CHAP in the beads, excluding the polymer dust (0.4% of the total batch) and the large agglomerates of fraction 1 (5% of the total), was of the order of 35 p.p.m. The concentration of residual CHAP, determined gravimetrically in a sample of commercial polystyrene beads, was about 15 p.p.m.

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### Synopsis

To determine the concentration of calcium hydroxyapatite occluded in polystyrene beads, styrene was polymerized in aqueous dispersion in the presence of calcium hydroxyapatite labeled with P<sup>32</sup>. It was found that the large beads contain a larger concentration of occluded apatite than the smaller ones. The results suggest that larger beads are formed during the "sticky stage" by coalescence of smaller ones carrying apatite imbedded in their surface.

### Résumé

En vue de déterminer la concentration d'apatite calcique occluse dans des perles de polystyrène on a polymérisé le styrène en émulsion aqueuse en présence de cette apatite calcique marquée au phosphore 32. On a trouvé que les grosses perles contiennent une plus grande concentration d'apatite occluse que les petites. Ces résultats suggèrent que durant l'étape visqueuse les grosses perles sont formées par le coalescence de perles plus petites porteuses d'apatite incorporée à leur surface.

### Zusammenfassung

Um die Konzentration des in Polystyrolperlen eingeschlossenen Calciumapatits zu bestimmen, wurde Styrol in wässriger Dispersion bei Gegenwart von P<sup>32</sup>-markiertem Calciumapatit polymerisiert. Es wurde gefunden, dass die grossen Perlen eine grössere Konzentration an eingeschlossenem Apatit enthalten als die kleineren. Diese Ergebnisse lassen vermuten, dass die grösseren Perlen während des "klebrigen Zustandes" durch die Vereinigung kleinerer entstehen, die in ihre Oberfläche Apatit eingebettet haben.

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