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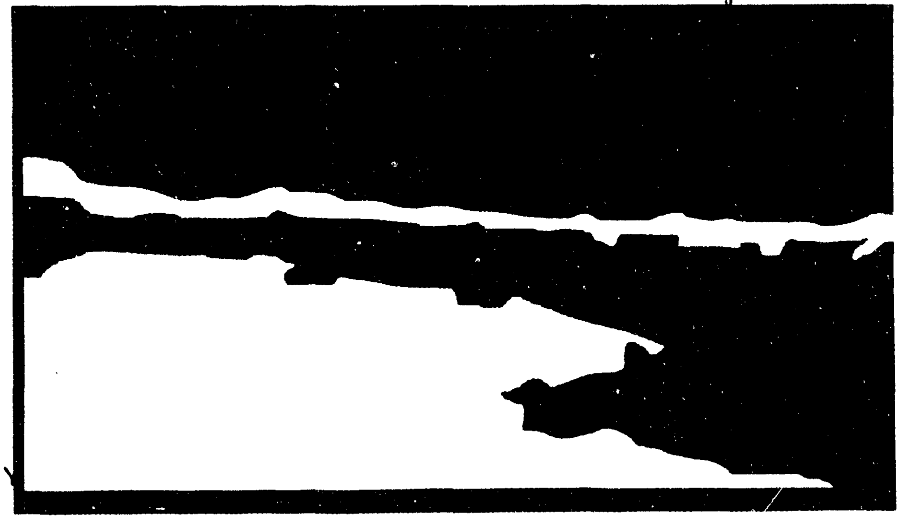

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APPLICATION OF SEPHADEX TO RADIOCHEMICAL SEPARATIONS

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Heteropolymolybdic acid species of a variety of elements bind to Sephadex (dextran cross-linked by epichlorohydrin). Solution pH can be used as a variable to affect the formation of heteropolymolybdates and thus their affinity for Sephadex. Therefore this material may be used to chromatographically separate certain elements which have been reacted with molybdate in solution. In this paper we report the measurement of distribution coefficients on Sephadex for heteropolymolybdates of As, P, Ge, Si, and Se from solutions of varying pH. We also describe the application of the technique to the recovery of Si-32 from proton-irradiated KCl targets and Ge-68 from proton-irradiated molybdenum targets.

INTRODUCTION

For two decades the Radioisotope Program at Los Alamos National Laboratory has used the 800 MeV, 1 mA proton beam at the Los Alamos Meson Physics Facility (LAMPF) to produce radioisotopes by spallation in selected target materials.[1] The recovery of the desired radioisotopic fractions represents a challenging separations problem because the radioisotopes are produced in very low quantities, ranging from nanograms to micrograms, in a target material weighing tens to hundreds of grams. This task is simplified when highly selective separation methods are available to isolate the radioisotopes from the target material. Chromatographic methods rely upon differential affinity of species for a stationary support phase to effect a separation when eluted with a selected mobile phase. The quality of an expected separation is often determined by inspection of available distribution coefficient data. In this paper we report the measurement of distribution coefficients on Sephadex (dextran cross-linked by epichlorohydrin) for heteropolymolybdates of As, P, Ge, Si, and Se from solutions of varying pH. We also describe the application of the technique to the recovery of Si-32 from proton-irradiated KCl targets and Ge-68 from proton-irradiated molybdenum targets.

For nearly 100 years, probably beginning with work by A. Jolles and F. Neurath, the unique properties of heteropolymolybdic and heteropolymolybdous species have been exploited extensively in analytical chemistry.[2] N. Yoza observed and studied the unique adsorptive behavior of polymolybdophosphates on Sephadex.[3] This work stimulated some recent novel analytical applications for concentrating and quantitating elements that form heteropolymolybdic species.[4,5] It was this that inspired us to develop applications of Sephadex to the recovery of Si-32 and Ge-68.

As we developed these methods we became interested in quantitatively evaluating the chromatographic potential of Sephadex because our target solutions contained heteropolymolybdate species of several elements. Solution pH is a major parameter affecting the formation of the heteromolybdate polymers, so our investigation focused on measurement of distribution coefficients for different elements as a function of pH. We use the term distribution coefficient throughout this paper to define a quantitative measurement of the fractionation of a heteropolymolybdate compound between Sephadex and a solution when the materials are stirred together. The number is calculated using the classic definition of distribution coefficient. It is important to remember however, that what is being measured is the affinity of the complex for the Sephadex, and that the pH effect that is observed is probably related more to the dynamics of the polymer formation than it is to a true reversible equilibrium between the polymer and the Sephadex. In future experiments we will be evaluating the reversibility of the sorption process.

EXPERIMENTAL

Materials: Except where noted, all reagents used in this work were analytical reagent grade or better. Water used in preparing aqueous solutions was obtained from a Barnstead Model D4741 purification system and had a resistivity of 17.5 MΩ-cm. We used Sigma LH-20 Sephadex. Radiotracers used in the work were provided by the Los Alamos Radioisotope Distribution Program, with the exception of P-32 which was purchased from Dupont. The Ge-68 was provided as Ge(IV) in a 0.5 F HCl solution. Arsenic-73 came as As(V) in a 0.1 F HCl solution. Selenium-75 existed as a mixture of Se(VI) and Se(IV) in 0.1 F HCl. Phosphorus-32 was obtained as sodium monohydrogenphosphate in water. Silicon distribution coefficients were measured using natural stable silicon obtained from stock standard solutions prepared using sodium hexafluorosilicate. The potassium chloride target material was of ultra-high purity obtained from Johnson Matthey Specialty Products. Molybdenum targets are made using metal having a molybdenum content of greater than 99%.

Analytical Equipment: All radiometric measurements were made using an EG&G Ortec model GMX-35200-S high purity germanium detector and Canberra Model Series 35-plus multichannel analyzer. Silicon colorimetric analyses were done using a Hewlett Packard Model 8452A Diode Array Spectrometer. Measurements of pH were done using a Beckman Model Φ40 pH meter.

Procedures:

Recovery of Si-32 from Proton-Irradiated KCl

A typical KCl target is made by melting 90-100 grams of potassium chloride and pouring the melt into a stainless steel container having roughly the size and shape of a hockey puck. The container is made by cutting a piece of 8 cm diameter stainless steel pipe to a length of about 2.5 cm and welding stainless steel face plates on each end of the pipe. The wall thickness of the container is about 0.3 cm. A hole is drilled into the side of the container through which the molten KCl is poured, and a stainless plug is welded in place to seal this hole. The target is encapsulated in an aluminum carrier, transported to the LAMPF

Isotope Production Facility (IPF), loaded onto a stringer, and put into the beam for irradiation. The carrier, IPF, and stringer assembly have been described elsewhere.[6,7] The two targets processed for Si-32 using the method described herein had an integrated exposure of 580,000 and 1240000 μA-hours. After the irradiation shorter lived isotopes are allowed to decay for 2-4 years before processing for Al-26 and Si-32.

Processing of targets begins by opening the encapsulation in a remote handling hot-cell facility, transferring the KCl to a dissolution vessel, and adding aqueous solvent. (We used 0.1 F KOH for the first target we processed and 0.1 F HCl for the second as we altered slightly our approach to obtaining Al-26 from the second target solution before recovery of the Si-32.) The resulting solution is pulled under vacuum through a 0.45 micron cellulose nitrate filter (Nalgene #245-0045). The filter and insoluble residues are taken to a radiochemistry hood for recovery of Al-26.[8]

The KCl solution filtrate, containing Si-32, Na-22 and traces of other long-lived radioisotopes is adjusted to a pH of between 1 and 2 using HCl or KOH as necessary. Silicon-free ammonium molybdate solution (0.045 F in molybdenum, pH = 1.75) is added in sufficient quantity to assure a stoichiometric excess for the formation of heteropolysiclicomolybdates. For silicon, stoichiometry requires 12 molybdenum atoms per silicon atom.[9] After addition of the molybdate, the pH is again checked to assure that it is between 1 and 2. The solution is then stirred for at least 20 minutes to assure complete reaction.

A 10 mL bed-volume of Sephadex (LH-20 in water) is prepared in a 2 cm diameter plastic column containing a coarse porosity plastic frit. The column is washed with 20-30 mL of solution containing sodium chloride at a concentration of 25 g/L and having a pH of 2. The salt is added to the solution because we have found empirically that its presence greatly increases the affinity of the silicomolybdate for the Sephadex. The target solution is transferred to this column and the silicomolybdic acid polymers are retained by the Sephadex as the eluate passes through the column. The column is then incrementally washed with 0.01 F HCl solution containing NaCl at a concentration of 25 g/L NaCl until Na-22 is no longer observed in the eluate. Ten mL of 0.4 F NaOH is loaded on the column and allowed to penetrate into the Sephadex bed to about 1 cm below the observed colored band, then allowed to sit for about 16 hours to decompose the heteropolymers of molybdate. After allowing the base to drain to the top of the column, we then wash with a 0.01 F HCl solution containing 25 g NaCl/L until Si-32 is no longer eluting. Residual molybdenum is removed from the radionuclidically clean Si-32 fraction by converting it to 4.5 F HCl, adding a trace of H_2O_2 and passing it through a 3 mL AG-1 X8 anion resin (100-200 mesh, chloride form) column. The molybdate is retained by the column and the Si-32 is eluted. The eluate is evaporated to soft dryness and reconstituted in 0.1 F NaOH to yield the product solution.

In this process we discovered that if Ge-68 is present, the bulk of the Si-32 elutes before the Ge-68 begins to elute. It was this observation that prompted us to measure the distribution coefficients of various heteropolymolybdate species on Sephadex because it appeared that chromatographic separation on the material was feasible. Knowing such distribution coefficients would permit the development of separation strategies using Sephadex chromatographically.

Recovery Ge-68 Product from Proton-Irradiated Molybdenum

A typical molybdenum target consists of about 350 grams of 2.5 cm diameter discs punched from 10 mil molybdenum metal foil arranged in 7 stacks in a stainless canteen the same size and shape as that described above for the KCl target. A molybdenum target receives about 350000 µA-hours of proton beam exposure. The primary isotope product recovered from these targets is Sr-82/[illegible]-82. The process begins by dissolution of the molybdenum metal in 30% H_2O_2. The dissolution generates 8-16 liters of molybdate target solution containing many radioisotopes in millicurie to curie quantities. Table 1 lists these isotopes.

The solution is first passed through a cation exchange column to remove the Sr-82 which is subsequently recovered and purfied.[10] The eluate from this column is processed for Ge-68 as follows. Sodium chloride is added to 500-750 mL aliquots of the solution to give a concentration of about 25 g/L and pH is checked to assure it is between 1 and 2, and adjusted if necessary. The solution is boiled to drive off excess hydrogen peroxide with resulting precipitation of some molybdous acid. The solution is filtered under gravity flow through a paper coffee filter. The filtrate is poured through a 50 mL bed volume Sephadex column, having a diameter of about 2.5 cm, that has been conditioned with a solution of 25 g NaCl/L having a pH of 2. Once the entire target solution has been treated the Sephadex is transferred to a 1 L Erlenmeyer flask with about 400 mL 6 F HCl. About half the volume of this solution is distilled into a trap vessel containing 10 mL 3% H_2O_2. The trap solution, containing Ge-68 and some arsenic radioisotopes is transferred to another container and adjusted to 9 F in HCl by addition of concentrated HCl. This solution is extracted with about 105 mL of chilled carbon tetrachloride to selectively remove the Ge-68. The Ge-68 is finally back-extracted into 5-6 mL of chilled water, yielding a product solution having a concentration of less than 0.5 F in HCl.

Measurement of Distribution Coefficients

Radiotracers were used to determine the distribution coefficients for molybdate polymers of arsenic (As-73), germanium (Ge-68), phosphorus (P-32), and selenium (Se-75); the stable element was used for the silicon measurements. Table 2 lists the amount of material used for each radiotracer experiment. We used 25 µmoles of sodium hexafluorosilicate for each stable silicon experiment. The measurement of the distribution coefficients was done using the same procedure in triplicate for each element.

A stock molybdate reagent solution was prepare by dissolving 4 grams of $(NH)_4Mo_7O_{24}\cdot 4H_2O$ and 12.5 grams of NaCl in sufficient water to give 500 mL of solution. We prepared molybdate reagent solutions of incremental pH ranging from 1 to 7 by adding sodium hydroxide or hydrochloric acid as necessary to the stock molybdate solution. For each element, in a 60 mL polyethylene bottle, we added a known amount of tracer to 25 mL of molybdate reagent at each of the seven pH values. We mixed the resulting solution for 35 minutes for heteromolybdate polymer formation. We then added 2.5 grams of LH-20 Sephadex to each bottle and mixed for an additional one hour. The mixture was then filtered through a 0.45 micron cellulose nitrate filter (Nalgene #245-0045). The filtrates containing radiotracers were assayed for activity concentration. By difference, the amount of activity sorbed by the Sephadex was calculated.

We used stable material for the silicon measurements because the radiotracer available to us was Si-32 with its 2 week half-life P-32 daughter. Both of these isotopes are beta emitters, and it was not possible for us to accurately measure silicon concentrations after the equilibration with Sephadex using radioanalytical instrumentation available to us without awaiting secular equilibrium between the Si-32 and P-32. Thus we used a spectrophotometric analytical procedure to measure the silicon concentration in the filtrates from the silicon experiments. We found that for accurate measurement it was necessary for us to first remove the molybdenum from the filtrate and convert to a near-neutral medium to coincide with our stock standard solutions used in the analysis. An analyte sample is prepared by adding concentrated HCl to 5 mL of filtrate to give an HCl concentration of 4.5 $\underline{F}$. This destroys the silicomolybdate polymers and provides a medium out of which the molybdate can be selectively sorbed on anion exchange resin. We add 10 microliters of 30% H_2O_2 to oxidize molybdenum to Mo(VI). The resulting solution is passed through a 2 mL column of AG 1X8 anion resin (100-200 mesh, chloride form) sorbing the molybdate and passing the silicon in the eluate. Sodium hydroxide is added to the eluate to bring the pH into the range of 2-9 before analysis. Samples were analyzed for silicon spectrophotometrically in accord with the method developed by Brzezinski and Nelson for dissolved silicon analysis of sea water samples.[5]

A stock silicon standard at 2.5 µmole/mL was prepared by dissolving 0.4702 g Na_2SiF_6/liter of solution. Metol-sulfite reagent was made by dissolving 6 g of sodium sulfite in 400 mL of water followed by dissolution of 10 g of paramethylamino sulfate (Metol). A reducing reagent solution was made by mixing 4.0 mL of Metol-sulfite solution, 3.2 mL water, 2.4 mL 9 $\underline{F}$ H_2SO_4 and 2.4 mL saturated oxalic acid solution. Calibration standards of 0, 5.0, 10, 20, and 40 micromolar were prepared by mixing 10 mL aliquots of salt solution (90 g NaCl/L water) with the necessary volume of silicon stock standard. Analyte samples were made by mixing 0.5 mL of the filtrate from the Sephadex with 5.0 mL of the salt solution.

Analysis was done as follows. Into individual plastic test tubes we dispensed 1.5 mL of each calibration standard and each analyte sample. To each tube we then added 0.6 mL ammonium molybdate reagent (0.045 F) and allowed 10 minutes for silicomolybdic acid polymers to form. We then added 0.9 mL of the reducing reagent and allowed about 3 hours reaction time as blue silicomolybdous acid species formed. The absorbances of the standards and samples were then measured at 810 nm on the HP diode array spectrometer. Concentrations of analyte samples were determined from the generated calibration curve.

RESULTS

SILICON-32 RECOVERY FROM KCl

Table 3 summarizes information on the two KCl targets that we have processed for recovery of Si-32. The average yield factor for Si-32 based upon these two target processes is 1.0×10^{-6} µCi/g KCl/µA-hr. The cause of the large disparity between the two specific activity numbers in Table 3 is explained in the discussion section below.

GERMANIUM-68 RECOVERY FROM MOLYBDENUM

We have processed 8 molybdenum targets for Ge-68 using the procedure described in this paper. Table 4 summarizes the data for these targets. The average yield factor is 1.7×10^{-6} mCi Ge-68/g Mo/µA-hr. Thus for a typical target (350 grams, 250000 µA-hrs exposure) we can recover about 150 mCi of Ge-68/Ga-68 having excellent radionuclidic purity (>99.9%). Because the final extraction volume is 5-6 mL, the concentration of activity in a typical batch is 25-30 mCi/mL decay corrected to end of irradiation.

DISTRIBUTION COEFFICIENTS

Table 5 shows the Sephadex distribution coefficient values for the heteropolymolybdates of the five elements measured in this work. The numbers given are the average of three trials at each pH. Relative precisions on these numbers range from 10-50%. The values for arsenic, germanium, phosphorus, and selenium were calculated based upon radioactivity as follows:

$$K_d = \frac{\mu\text{Ci radioelement} / \text{gram Sephadex}}{\mu\text{Ci radioelement} / \text{mL filtrate}}\ .$$

The values for silicon were determined using the equation:

$$K_d = \frac{\mu\text{mole silicon} / \text{gram Sephadex}}{\mu\text{mole silicon} / \text{mL filtrate}}\ .$$

The concentration of element in the filtrate was obtained by measurement and the amount per gram of Sephadex was then obtained by difference based upon the amount added to the original solution. Values listed as greater than 1.0 x 10^5 indicate that to the limits of detection there was no measurable element in the filtrate solution. Values of zero imply that, to the precision of our measurement, we found all of the material added in the original solution to still be present in the filtrate.

DISCUSSION

SILICON-32 FROM KCl

The yield of Si-32 is about the same for the two KCl targets processed to date when it is noted that target #56-20-1 had nearly twice the beam exposure of #51-20-1 and yielded twice the amount of Si-32. It should be noted, however, that in neither case do we know how efficient our recovery process was because we could not analyze for Si-32 in the original target solution due to the presence of relatively large quantities of Na-22. We plan to do some tracer experiments in mock KCl targets to determine what our process yields might be, but have not yet completed these experiments.

The specific activity of Si-32 from target #56-20-1 (47130 Bq/µg) is nearly 100 times greater than that from target 51-20-1 (518 Bq/µg). This is probably because the KCl target solution for 51-20-1 was processed for Al-26 before consideration of recovery of the Si-32 and no precautions were taken to prevent addition of stable silicon by process chemicals and equipment during the Al-26 process. For target #56-20-1 we took precautions during the whole process to protect the target solution from contamination with natural stable silicon. The only other report we found regarding Si-32 production was the work of Polak, et al..[11] They processed a proton-irradiated vanadium metal target for Si-32 recovering several hundred microcuries of Si-32, but with a very low specific activity reported to be only about 22 Bq/µg Si.

GERMANIUM-68 FROM MOLYBDENUM TARGETS

As with Si-32 from KCl, we have not been able to accurately evaluate the overall efficiency of the Sephadex process for the recovery of Ge-68 from the molybdenum targets. These targets are extremely rich in gamma-emitting isotopes, and due to the extent of dilution necessary to make an assay sample and the very low branching ratio for the 1077 keV gamma from the Ga-68 daughter which we use to assay our Ge-68 products, we cannot obtain an accurate pre-process value for the Ge-68 activity in the molybdate target solution. However, because of the large distribution coefficient for the polygermanomolybdate on the Sephadex under the conditions of our process, and based upon our experience with the distillation/extraction purification of Ge-68 from our RbBr targets,[12] we are confident that we recover the material in high yield (probably >90%). This is important because in recent years we have found it increasingly difficult to meet the demand for this isotope from our RbBr target irradiations alone. With other target commitments, it is not feasible to substantially increase our RbBr irradiation capacity. Thus because of the discovery of Ge-68 in our molybdenum targets, which are irradiated primarily to produce Sr-82, and the development of this process to recover the germanium from molybdenum we have been able to increase our Ge-68 production capacity so that we have continued to meet demand. The history of Ge-68 production at Los Alamos is discussed in more detail elsewhere.[13]

DISTRIBUTION COEFFICIENTS

The primary purpose of the work to measure the distribution coefficients as a function of pH was to evaluate the feasibility of chromatographically separating various heteropolymolybdates using Sephadex as the stationary support. Perusal of Table 5 would seem to indicate that with careful selection of mobile phase pH, it should be possible to cleanly separate some of these species on Sephadex. Just to cite one example, silicon, germanium and arsenic have significantly different retentions on Sephadex out of molybdate solutions at a pH of 4. The differences in retention are likely due to differences in extent of formation of polymer rather than differences of polymer affinity for the Sephadex.

We evaluated the behavior of selenium on Sephadex because there are curie quantities of Se-75 in the molybdate solution. It is known that Se(IV) will form significantly lower molecular weight heteropolymers with molybdate as compared to the other elements in our study.[9] It is apparent from our data that our Se-75 tracer is not retained to any significant extent under the conditions of our experiment. It may be that the bulk of the selenium is in the Se(VI) form, and thus the polymers are not forming. It may also be that the lighter polymers do not react in the same way as the heavier polymers with LH-20 Sephadex. We feel it would be useful to further explore the behavior of selenium.

Our experience with the recovery of Si-32 from the KCl target solution reveals an interesting anomaly in the context of the measured distribution coefficients. In target 56-20-1, after removal of Na-22, we observed nanocurie levels of Ge-68, formed by spallation in the stainless steel target container, present in the target solution. We also knew that under the conditions of our experiment the Ge-68 would be sorbed on the Sephadex along with the Si-32. After sorbing these on the Sephadex and washing away Na-22 with pH 2 saline solution, we intended to decompose the polysilicomolybdate complex with dilute sodium hydroxide to strip the Si-32 from the column. We expected to see the Ge-68 and the Si-32 come off the column together. However, we were able to wash the bulk of the Si-32 from the column as described in our procedure before the Ge-68 came off. In fact it was this observation that stimulated the measurement of the distribution coefficients. Interestingly, on the basis of the measured K_d values, if we would have predicted any chromatographic separation it would have been that the Ge-68 would elute first. The literature reveals that there are several kinetic and thermodynamic factors that will influence formation of the polymers and thus their retention by Sephadex,[14,15,16,17,18] and we believe it would be valuable to the field of radiochemical separation technology to further systematically evaluate these factors in the context of chromatographic separations.

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TABLE 1.

Gamma-emitting Radioisotopes Identified in Molybdenum Target Solution

Tc-97m	Tc-95m	Nb-95	Nb-92
Zr-95	Zr-88	Y-88	Sr-85
Sr-82	Rb-84	Rb-83	Se-75
Se-72	As-74	As-73	As-72
Ge-68	Zn-65	Co-56	Co-57
Co-58	Fe-59	Mn-54	Cr-51
V-48	Sc-46	Na-22	Be-7

TABLE 2.

Radiotracers Used for K_d Experiments

Radiotracer	Activity per Experiment
As-73	0.71 μCi
Ge-68	3.65 μCi
P-32	1.68 μCi
Se-75	0.48 μCi

TABLE 3.

Potassium Chloride Target Data.

Target #	Target Mass (g)	Integrated Exposure (μA-hrs)	Yield (μCi)	Specific Activity (Bq/μg)
51-20-1	94	576853	60	518
56-20-1	98	1239000	110	47130

TABLE 4.

Molybdenum Target Data.

Target #	Target Mass (grams)	Integrated Exposure (µA-hrs)	EOB Yield mCi	Yield Factor mCi/g/µA-hr
3-30-92	80	2.72×10^{-5}	45	2.10×10^{-6}
92-1-3	351	3.23×10^{-5}	228	2.00×10^{-6}
92-1-4	353	2.04×10^{-5}	160	2.20×10^{-6}
92-1-1	334	3.88×10^{-5}	146	1.10×10^{-6}
92-1-2	346	1.11×10^{-5}	60	1.60×10^{-6}
92-1-5	363	1.72×10^{-5}	100	1.60×10^{-6}
92-1-6	385	3.49×10^{-5}	100	8.00×10^{-7}
92-1-7e	470	1.99×10^{-5}	258	2.80×10^{-6}

TABLE 5.

Distribution Coefficients for Heteropolymolybdates on Sephadex

pH	Arsenic	Germanium	Phosphorus	Silicon	Selenium
1	4.2×10^{4}	$>1.0 \times 10^{5}$	4.4×10^{3}	$>1.0 \times 10^{5}$	2.2
2	2.0×10^{4}	$>1.0 \times 10^{5}$	9.0×10^{3}	$>1.0 \times 10^{5}$	0.5
3	34	2.5×10^{4}	6.2×10^{3}	$>1.0 \times 10^{5}$	0.6
4	3.0	170	1.0×10^{3}	$>1.0 \times 10^{5}$	0.7
5	3.7	6.4	6.0	2.5	0
6	3.3	0.2	0.5	0.4	0
7	2.5	0.3	0.7	0.6	0

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