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A RAPID DIFFUSION METHOD FOR PHYSICOCHEMICAL CHARACTERIZATION OF METAL LIGANDS IN SOILS AND SEDIMENTS

by T. R. Garland, R. E. Wildung, and R. A. Pelroy

Battelle
Pacific Northwest Laboratories
Richland, Washington 99352

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THE BIOLOGICAL IMPLICATIONS OF METALS IN THE ENVIRONMENT--SESSION II

A RAPID DIFFUSION METHOD FOR PHYSICOCHEMICAL CHARACTERIZATION OF METAL LIGANDS IN SOILS AND SEDIMENTS

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The ability to predict the effect of the addition of any pollutant on a complex ecosystem requires a thorough knowledge and understanding of the interactions of the components of the system at the chemical level as well as the organism level. In the case of metals in soils and sediments, the major fraction of the exogenous metal is usually immobilized and only a small fraction may be available to biota in the ecosystem. It is, therefore, logical that considerable emphasis has recently been devoted to determination of the chemical form of "mobile" or "soluble" metals in soils and sediments, especially those that have the potential for adverse effects on biota at relatively low concentrations.

However, it is extremely difficult to determine the chemical form of most metals at environmental levels. In addition to difficulties inherent in detection of metals at low levels, methods of metal isolation and characterization have the inherent limitation of potentially changing the chemical form initially present in situ.

Today, a diffusion method will be described which allows preliminary characterization of mobile species of metals in soils and sediments while minimizing the potential for alteration of metal form. In addition, examples of the application of the method to model compounds of Pu and Pu in soil will be given.

The impetus to develop and utilize diffusion phenomena as an analytical tool came from microbial studies in which diffusion was used to create metal concentration gradients on agar-gel plates to expose metal-resistant microorganisms to different metal concentrations on a single plate. It was noted that the application of the most simple equations describing diffusion allowed the accurate prediction of the mobility, as a function of time, of known aqueous metal species in aqueous solution.

The first slide shows two plates which have been employed for measuring the diffusion of mobile metal species in soils and sediments.

SLIDE 1:

This slide shows the petri plate and tube container and an example of the sampling procedure used. The supporting medium used in both systems is a 2% agar gel in 0.01 M $Ca(NO_3)_2$, which stabilizes the aqueous phase against convection but does not impede the random movement of most molecules at dilute concentrations.

When the petri plate system is employed, the soil sample under study is applied in a cylindrical well at the center of the plate. Radial diffusion of soluble substances occurs in a plane which is sampled with time. When the tube system is employed, the sample is applied at the surface of the gel and diffusion occurs in one direction. The dimensions of the containers are not critical except that they should be of sufficient length (or radius) that wall effects are negligible during the sampling time period. This is usually not a significant problem and is easily recognized as will be discussed later. Precautions must be taken to assure that no seepage of sample occurs between the gel and the sides of the container. This may entail sealing the sample well with additional agar solution prior to the application

of the sample or pretreatment of the container. In the latter case, preliminary addition of hot agar to the container, decantation of excess agar which did not adhere to the glass followed by cooling and drying in a desiccator prior to addition of the final gel matrix has proved to be sufficient to allow bonding of the agar to plastic or glass.

The remainder of the discussion will concern only the petri plate; however, only slight changes in mathematical and procedural changes are required for the tubes.

Preparation of the plates is accomplished several days in advance of the addition of samples. In order to obtain reproducible volumes in the petri plates, a known quantity of hot 2% agar in .01 M $Ca(NO_3)_2$ is weighed into pretared petri dishes and allowed to solidify on a level table. Usually, several plates are made at one time, and plates not being immediately used are stored at 4°C. The agar plates are equilibrated in a constant temperature chamber for 24 hours.

Insertion of samples into the agar containers has been accomplished using various techniques. Prior to placement of soil in the diffusion chamber, a 10 g subsample is taken to field moisture capacity. Using a pretared segment of glass tubing with an inside diameter the equivalent to the well, a plug of the moisture-saturated soil is removed, weighed, and inserted into the well. The amount placed into the agar is obtained by difference. A separate soil sample is taken in an identical matter to accurately determine (gravimetrically) the soil moisture content.

After the soil has been added to the agar gel, it is gently tamped to assure uniform contact with the gel surface. Prior to incubation, the sample is sealed with approximately 0.5 ml of 0.7% agar at a temperature

of 45°C (the lowest temperature at which agar is not solidified) to eliminate differential drying of the sample and gel.

The sample in the agar gel diffusion container is incubated for predetermined periods of time at constant temperature. The incubation is generally carried out inside a large vacuum dessicator with a damp sponge added to maintain constant moisture. The dessicator arrangement also allows incubation under aerobic or anaerobic conditions by changing the atmosphere in the chamber. The dessicator is then stored level in a constant temperature incubator at the desired temperature.

Samples of the agar gel on petri plates are taken for analyses of the desired metal at definite time intervals by removing small cylindrical samples at specific distances from the sample well. With the petri plates, several samplings on the same plate may be undertaken at different time intervals. For the tubes of agar gel, each sampling requires the sacrifice of a replicate tube and the sample.

The next slide shows the mathematical equations used to describe the movement of solutes by diffusion in the supporting gel matrix for the petri plate system.

SLIDE 2:

The two-dimensional planar diffusion on the petri plates is represented by the first equation where C is the concentration in $\mu g/cm^3$ at any point in the agar at distance 4, S is the quantity of material at t_o in the starting material, D is the diffusion coefficient of the material, t is Δt, i.e., $t - t_o$ in sec, and h is the height of the agar layer in cm.

The diffusion coefficient is determined from the slope of the line obtained from equation 1 using equation 2. This equation is derived by

taking the logarithms of the first equation and differentiating with respect to r^2. Equation 3 is an approximation relating the diffusion coefficient to the square root of the molecular weight of a known and unknown species. Several assumptions have been made in the use of these equations. First, it is assumed that the sample has a negligble thickness, i.e., it represents a line source. To minimize this limitation, the diameter of the plug was maintained as small as possible and the distance and time chosen for analyses were maintained as large as possible. Secondly, it is assured that the substances move by diffusion processes alone and do not interact with the matrix or other components during the time of analyses. This later limitation has been shown to be a problem and is likely the major limitation in the interpretation of results obtained. To assist in recognizing when interactions with the gel have occurred, diffusion coefficients and calculated metal concentrations are compared at different time intervals after sample application.

The next slide shows a plot of the diffusion of a stable complex of Pu_2DTPA_3 in the petri plate chamber.

SLIDE 3:

The log of the concentration of Pu at time t and distance r from the original sample in µg/ml is plotted on the y axis and the square of the distance traveled is plotted on the x axis. The results are for Pu at levels of 40 µg and 0.04 µg, the solid and dashed lines, respectively; and at two time intervals, 24 and 94 hr from sample application. The straight and parallel lines obtained for both concentrations and at both time periods are an indication that sample or gel interactions are not occurring.

The consistent diffusion coefficients and the comparability of initial and calculated initial Pu concentrations for different equilibration times at different concentrations verifies this observation. The diffusion coefficient observed for this stable complex of Pu was 5.5×10^{-6} cm^2/sec.

Two primary mechanisms for achieving stability in Pu aqueous solution (including the soil solutions) are likely. One involves complexation with organic ligands to form highly stable chelates or complexes as with DTPA, where the log stability constant is in the vicinity of 23, and the other involves hydrolysis to the highly insoluble hydrous oxides or hydroxides, with log solubility products approaching 50. Several studies underway in our laboratory and others are attempting to define the plant uptake of Pu after addition of $Pu(NO_3)_4$ solutions to soil where the Pu is stabilized in solutions of 2 $\underline{M}$ HNO_3. In our laboratory, the neutralization of the excess acid has been accomplished by the addition of sufficient $CaCO_3$ to neutralize the acid which had been added to a small aliquot of soil. The amended soil was then dried and mixed with the remaining soil required for the experiment. To characterize the Pu reactions, products which may have formed during this procedure, the diffusion of $Pu(NO_3)$ added to an agar solution which contained sufficient $CaCO_3$ or $Ca(OH)_2$ to neutralize the excess associated acid was measured. After stirring for 5 min, the agar solutions containing the Pu were allowed to solidify and equilibrate for 24 hr. The final pH of both solutions approximated 8 as indicated by pH paper. After equilibration, a subsample of the solidified agar was added to another prepared agar plate for diffusion measurements after 94 and 438 hr incubation. The concentration of Pu was 0.1 $\mu g/cm^3$ for both cases.

The results of this experiment are shown in the next slide.

SLIDE 4:

The Pu species behaved differently than the Pu_2DTPA_3 complex. First, the lines are not linear and second, the diffusion coefficients for Pu are considerably smaller. It is also of interest that more Pu appears to be able to diffuse from the plug when neutralization of the excess acid was made with $Ca(OH)_2$ than with $CaCO_3$. Our tentative interpretation of this data is that the majority of the Pu that appears to be diffusible, is primarily in the form of colloidal hydrous oxide particles of Pu with estimated molecular weights of 200,000 to 1 million. However, it is not impossible that a combination of colloidal $Pu(OH)_4$ and stable Pu-ligand complexes (where the organic ligand was an impurity in the agar) is responsible for the observed behavior. This will be discussed later. The next slide shows the diffusion of Pu form a representative soil which had $Pu(NO_3)_4$ added with the excess acid neutralized with $CaCO_3$ but which had been allowed to incubate under aerobic conditions for approximately 100 days at 70% field moisture capacity prior to diffusion measurements.

SLIDE 5:

This slide includes, for comparative purposes, the diffusion of Pu on agar gel petri plates (dashed lines), as shown in the previous slide. In addition, the behavior of Pu in the equilibrated soil (solid line) is also shown. The Pu analyses were conducted at two time-intervals, i.e., 94 hr (open circles) and 438 hr (closed circles). This particular soil is a Ritzville silt loam, and the observed behavior of Pu on this soil is generally representative of that found on four other soils. As indicated earlier, the nonlinear semi-log plots obtained may be explained by either multiple chemical species of Pu of different diffusion coefficients giving

the composite curves shown or by interaction of Pu with the agar gel matrix. Investigations currently in progress involve the use of computer analyses of diffusion curves to resolve the multiple diffusing species and to differentiate between the diffusible and the interacting, nondiffusible species. For the purpose of this discussion, diffusion coefficients, sample concentration, and molecular weights have been estimated using the data points at the extremes of each curve. These values will be summarized later.

The curves shown here and those obtained from experiments with the other soils lead to several conclusions. First, the magnitude of the difference in the quantity of diffusible Pu resulting from solution neutralization and neutralization in the soil likely results from differences in aging time after neutralization and sorption in the soil systems. In the solution studies, the reaction time after neutralization was only 24 hr compared to the 100 day incubation for the soils studies. The $Pu(OH)_4$ formed by hydrolysis or neutralization tends to agglomerate into larger particles with time with the rate being at least partially Pu-concentration dependent. Secondly, the common slope of the chemical species formed in soil and in solution over the distance of 2 to 2.5 cm (or in terms of r^2 on the x axis, from 4 to 6) indicates the common presence of colloidal Pu hydroxide of sufficiently small size and electrostatic charge to move in both the aqueous soil-free solution and the soil solution. Thirdly, the reduced slopes at the extremes of the curves for diffusible soil Pu compared to solution Pu may indicate that a small fraction of the added Pu has associated with ligands present in soil forming a complex that is of sufficient stability to resist hydrolysis and immobilization.

SLIDE 6:

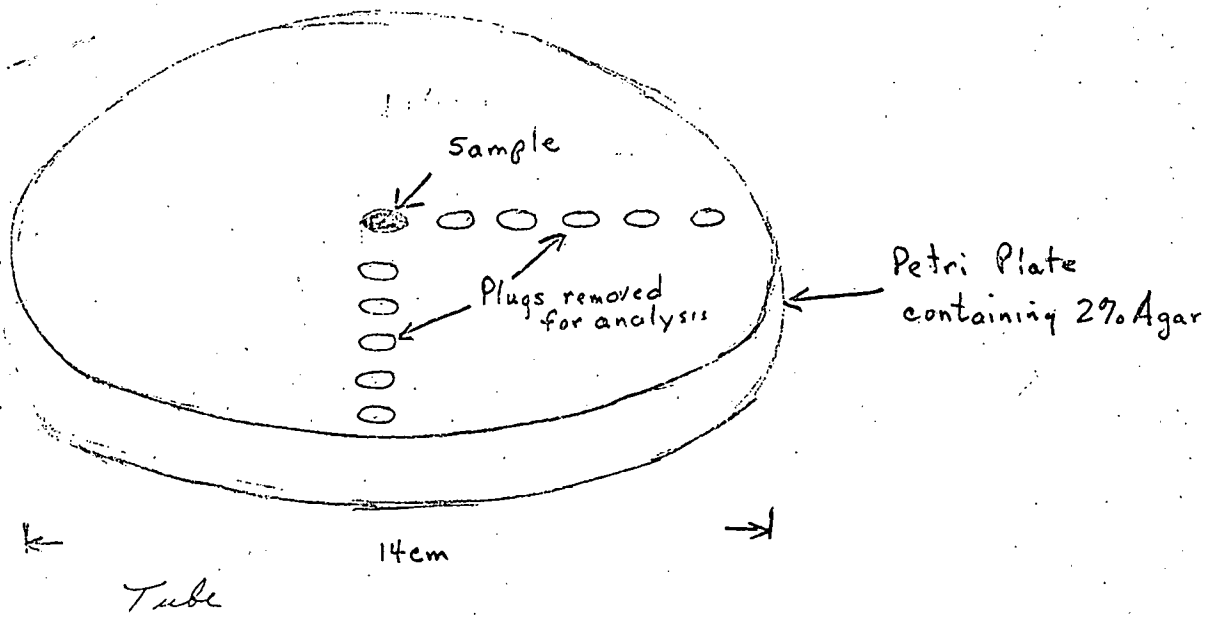
The result of these studies to date are preliminary to the extent that there has been no attempt to precisely resolve the several chemical species which serve as components of the diffusion curves. In this respect, the diffusion coefficients for the most mobile components are the least subject to error. The less mobile components (those with larger molecular weights) are more likely to have diffusion coefficients and concentrations that are lower than those shown.

Of particular importance are the estimated concentrations and molecular weights of the most mobile fractions of the Pu in the soils studied. Although the quantity of Pu associated with the most mobile fraction (9-55 pg/g soil) represents less than 0.008% of the Pu in the soil, this fraction may represent that component of Pu that is most available to plants. In fact, this approximates the quantity of Pu removed from soil by mature soybeans grown in pot culture in our laboratory.

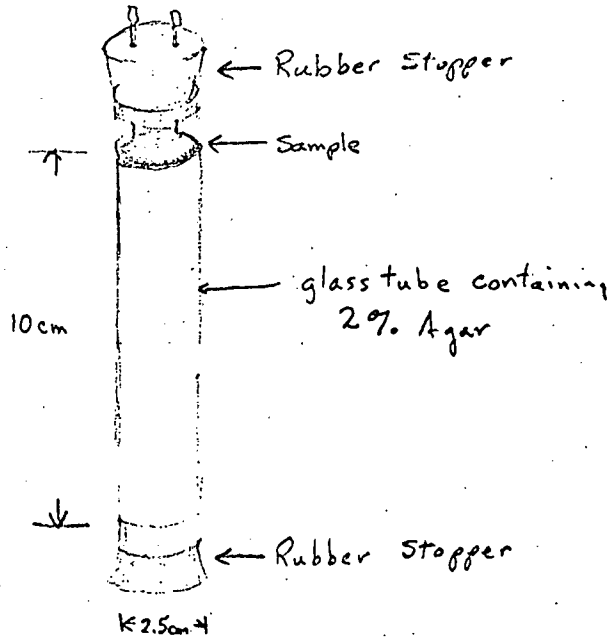
In conclusion, the diffusion method presented allows the preliminary characterization of mobile metal species present in soil solution and sediments without markedly affecting the form of the model compound. Furthermore, the liklihood of alteration of less stable Pu complexes is less than harsh chemical extracts. The addition to soil of Pu in acid solution with subsequent neutralization of the excess acid results in the immobilization of the majority of the Pu, but that a very small fraction that is relatively mobile with estimated molecular weights of less than 20,000. Application of this method to soil and sediment systems should provide insight into the chemical and microbiological phenomenon which influence Pu mobility and bioavailability in the environment.

Slide 1 Petri plate and Tube diffusion containers

Petri plate



Tube



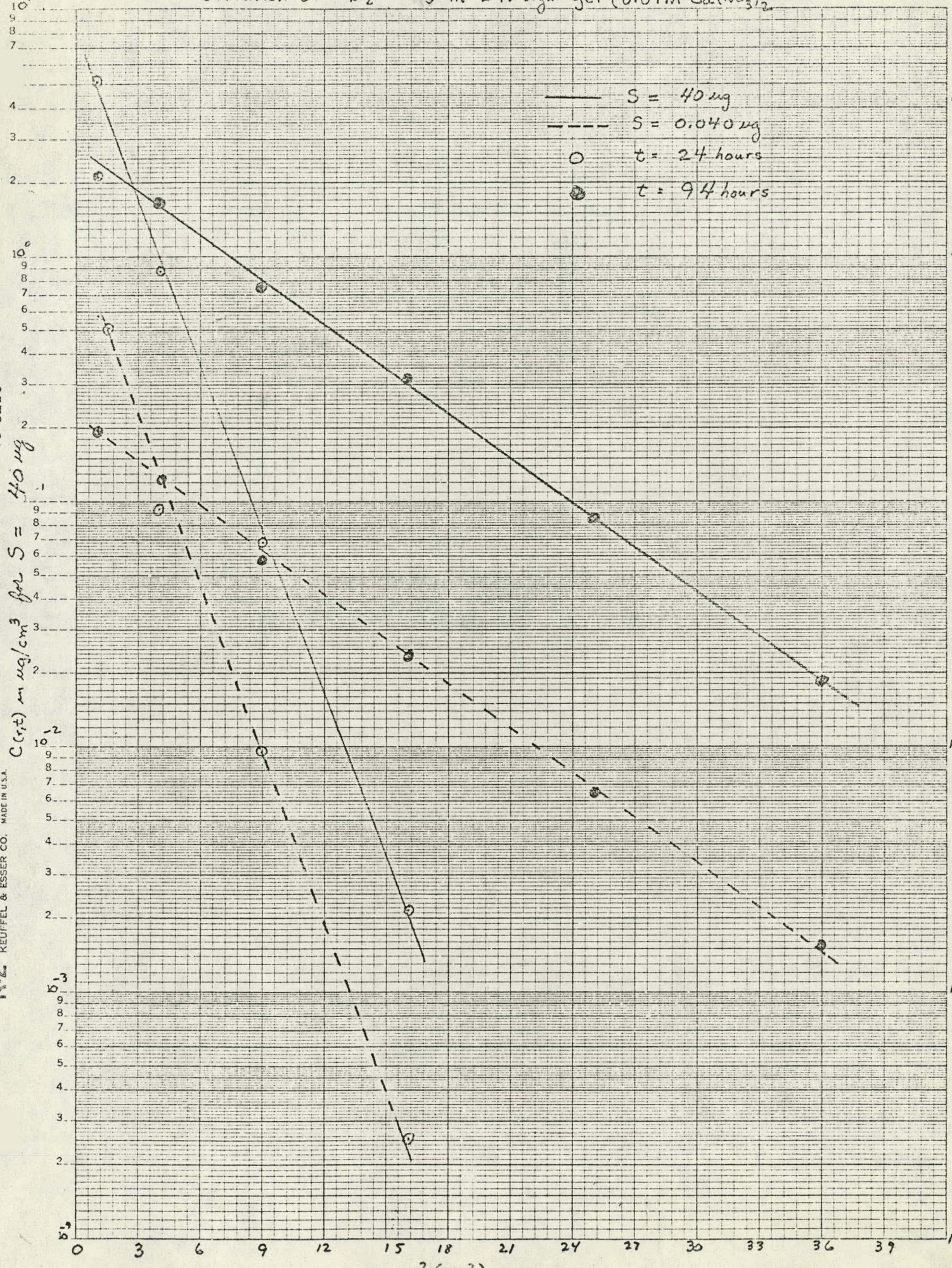
Slide 2. Equations used for analysis of metal diffusion on petri plates.

1. $$C(r,t) = \frac{S}{4\pi Dth} e^{\frac{r^2}{-4Dt}}$$

2 $$\frac{d}{d(r^2)} (\log_e C) = \text{slope at time}(t) = -\frac{1}{4Dt}$$

3. $$D_1 \sqrt{MW_1} = D_2 \sqrt{MW_2}$$

Slide 3. Diffusion of Pu_2DTPA_3 in 2% agar gel (0.01M $Ca(NO_3)_2$



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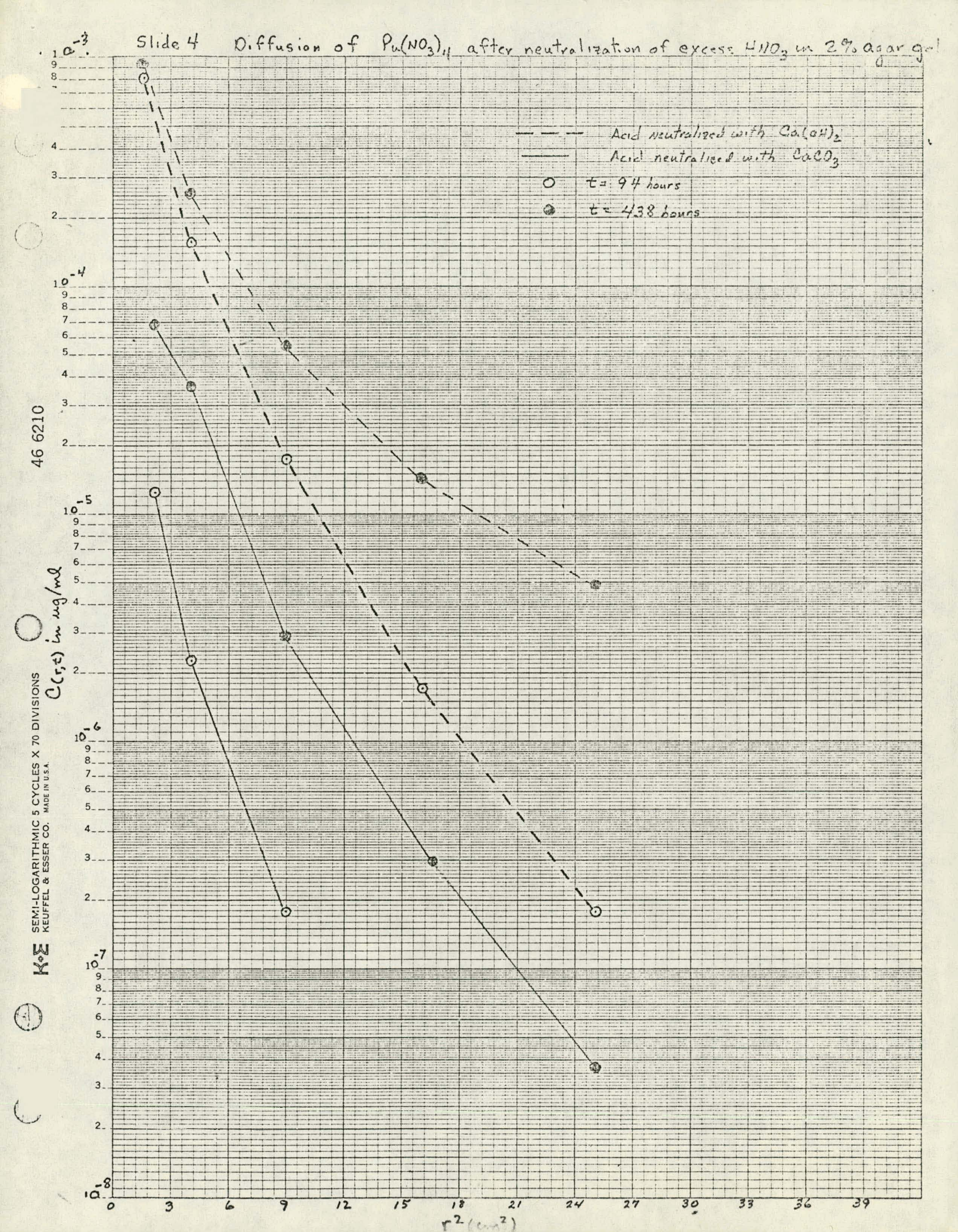
K+E SEMI-LOGARITHMIC 5 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

Slide 4 Diffusion of $Pu(NO_3)_4$ after neutralization of excess HNO_3 in 2% agar gel



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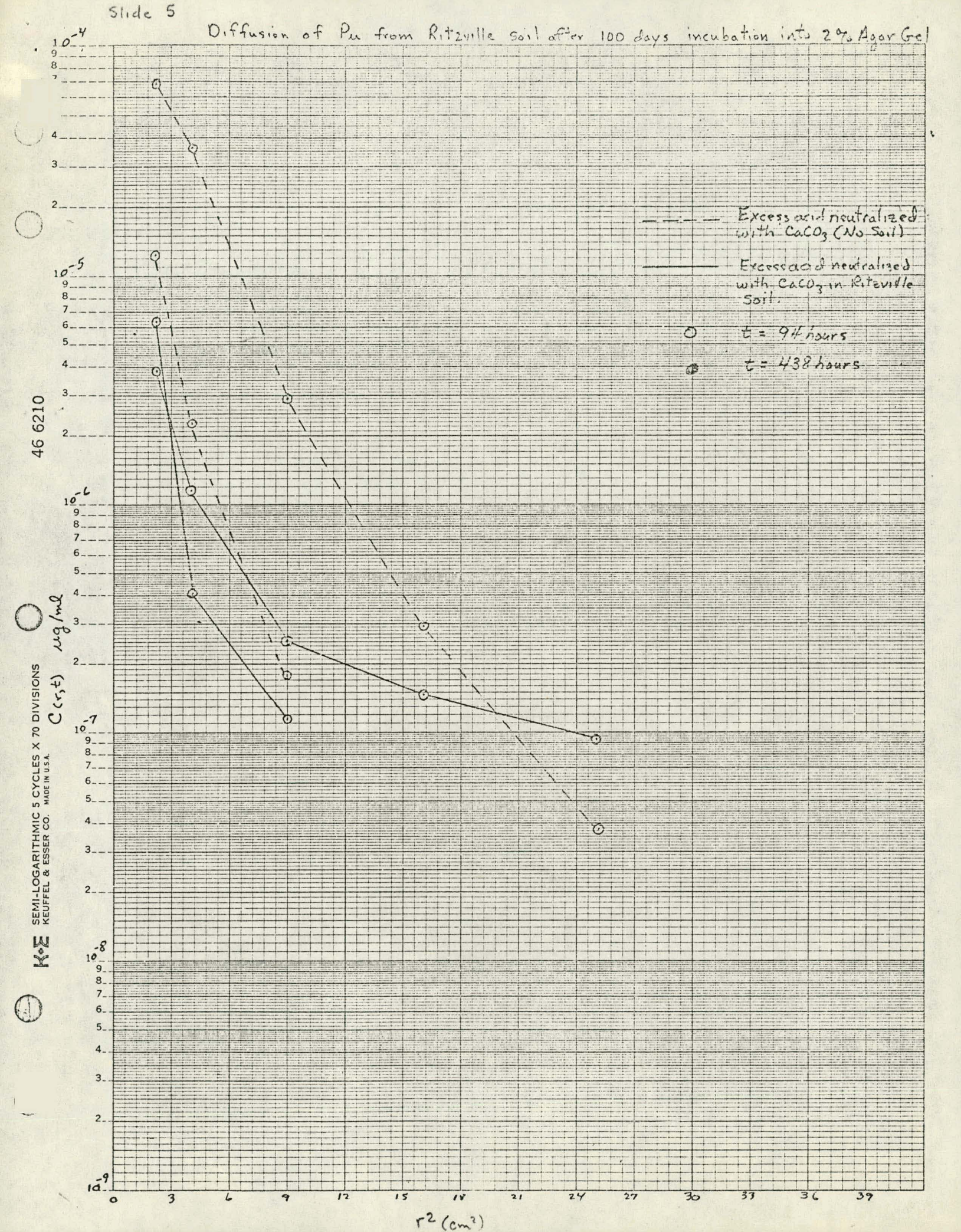
Slide 5


Diffusion of Pu from Ritzville soil after 100 days incubation into 2% Agar Gel
Excess acid neutralized with $CaCO_3$ (No Soil)
Excess acid neutralized with $CaCO_3$ in Riteville Soil.
t = 94 hours
t = 438 hours
C(r,t) μg/ml
r^2 (cm²)
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Slide 6. Estimated concentrations and molecular weights of "mobile" Pu ligands in soils from measured diffusion coefficients.

Identification	Most Mobile Species			Least Mobile Species		
	Diffusion Coefficient ($\times 10^{-6}$)	Molecular Weight	Soil Concentration pg/gm*	Diffusion Coefficient ($\times 10^{-7}$)	Molecular Weight	Soil Concentration pg/gm*
Controls						
$Pu(NO_3)_4$+ $Ca(OH)_2$	3.0	5,200	--	2.1	1×10^6	--
$Pu(NO_3)_4$+ $CaCO$	1.5	22,000	--	4.4	0.2×10^6	--
Pu_2DTPA_3	5.8	1,700	--	53	1,700	--
Soils						
Ritzville	3.0	5,000	24	2.3	0.9×10^6	150
Quillayute	2.5	7,200	47	2.7	0.7×10^6	1,200
Hesson	2.4	8,100	9	2.7	0.6×10^6	330
Salkum	1.5	21,000	55	2.3	0.8×10^6	340
Muscateen	1.9	13,000	36	3.1	0.5×10^6	170

* picograms (10^{-12}) Pu/gram soil.