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PREPARATION OF GALLIUM-68 RADIOPHARMACEUTICALS FOR POSITRON TOMOGRAPHY

Progress Report

for period

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REA

DISCLAIMER

NOTICE

ABSTRACT

Although the germanium-gallium generator is probably the only source of positron-emitting radionuclides that would enable the wide application of positron tomography, the generator system in use suffers from several major disadvantages. The most important of these is that the generator is eluted with EDTA, and EDTA forms a very strong chelate with gallium. In order to produce radiopharmaceuticals other than gallium-68 EDTA it is necessary to break the stable EDTA complex and remove all the EDTA. We have developed a new generator system using a solvent extraction system which will produce gallium-68 8-hydroxyquinoline, a weak chelate. Using this agent we have synthesized several gallium-68 radiopharmaceuticals and tested them in vitro and in vivo. Attempts have been made using polarographic and chromatographic techniques to investigate the stability of gallium-68 complexes with a series of cryptates.

OBJECTIVES

The aims of this project are to:

1. Develop a germanium-68/gallium-68 generator that does not produce gallium-68 as gallium-EDTA;
2. To use the product of this generator to produce a series of gallium-68 radiopharmaceuticals.

WORK IN PROGRESS

Work carried out on this project has centered on four areas:

1. Development of an improved germanium-gallium generator;
2. Preparation of radiopharmaceuticals using the gallium-68 from the generator;
3. Development of a general method for preparing gallium-68 labeled proteins;
4. Approaches to the production of lipophilic gallium-68 labeled compounds.

1. GALLIUM-68 GENERATOR

Over the past two years, we have developed a solvent extraction germanium-68/gallium-68 generator which provides gallium-68 in the form of ^{68}Ga-oxine. As we have discussed in the past, the ^{68}Ga-oxine is suitable as a precursor for the preparation of a series of ^{68}Ga radiopharmaceuticals. An automatic solvent extraction generator was fabricated in the Washington University Cyclotron machine shop during the period June 1977 to October 1978. The general plan of the generator is shown in Figure 1. Briefly, the initial extraction is performed in the upper

flask after addition of the oxine-ethanol solution, mixing, and addition of chloroform or methylene chloride. Mixing is accomplished using a stirring motor and paddle-rod, while phase separation is accomplished using a teflon ball floating on the phase interface which will intercept a photoelectric beam at the neck of the flask and thus cause the solenoid valve to shut. The organic ^{68}Ga-oxine phase will drain into a second flask containing an equal volume of buffer and inactive, carrier germanium where a backwash will be accomplished. The second phase separation is accomplished the same way as the first.

The electronics package necessary to time and control these operations is shown in Figure 2. Utilizing extensive integrated circuitry and all solid-state devices, this package provides for a 30 second premix cycle after the addition of the oxine-ethanol solution. Following that, chloroform is added and the full cycle is initiated. Phase mixing is done for 6 minutes, followed by a 1 minute pause for complete phase separation. The first solenoid valve is then opened; when the ball triggers the photoelectric cell the valve automatically closes. After a 30 second period to ensure complete drainage the second stirring motor operates for 2 minutes, followed by another 1 minute waiting period for complete phase separation. Drainage then begins and is halted when complete by a second floating ball. The organic phase is collected in a suitable vessel and evaporated to dryness manually.

Testing of the generator was initially carried out using a trial loading of 5 μCi of ^{68}Ge. The ^{68}Ga-oxine yields and ^{68}Ge breakthrough values for this test generator are shown in Table I. The overall yield of ^{68}Ga-oxine

is 55% with .001% ^{68}Ge breakthrough. After the first separation, the ^{68}Ga yield is 67% with .004% ^{68}Ge breakthrough. This is comparable to the 75% yield of ^{68}Ga that we have obtained from our prototype system using manual separations. It seems that only a small amount of ^{68}Ga is lost in the backwash and the ^{68}Ge breakthrough is reduced by a factor of 4 to a very low level. By comparison, ^{68}Ga-EDTA alumina columns exhibit yields of around 50% with ^{68}Ge breakthroughs of about .004%. These preliminary tests showed that the generator is fail safe, in no cases was the ^{68}Ge lost to the second flask. It should also be noted that if one of the light sources should fail, the corresponding valve would never open, and there would be no risk of spilling the aqueous ^{68}Ge. As the next phase of the testing, the generator was loaded with larger amounts of ^{68}Ge and attempts were made to prepare our routine series of radiopharmaceuticals. We have increased the size of the generator to greater than 12 mCi which is considerably larger than we have ever utilized in our prototype system. We have found problems in producing some of the radiopharmaceuticals, specifically the labeled platelets,utilizing this large generator. One possible explanation for this is that the high radiation dose from the ^{68}Ge decay causes so much damage to the siliconized glass containing the solution that metal ions are continuously released from the glass thus affecting the radiopharmaceutical production. We have therefore recently studied the separation of ^{68}Ga-oxine from ^{68}Ge in clear teflon separatory funnels. These funnels are available from several manufacturers and would be suitable for the automatic generator as they are transparent to light. Preliminary data obtained in the last few weeks leads us to believe that the problems

observed using the glass separatory funnels can be overcome utilizing the teflon ones and we hope in the next few months to convert the automatic generator to one which utilizes teflon funnels. It is also planned to eliminate the containment of the ^{68}Ga in glass completely and this will be carried out by performing the boiling down of the ^{68}Ga-oxine solution in teflon tubes. Although problems have been encountered in increasing the size of our automatic generator we have demonstrated that an automated ^{68}Ga solvent extraction generator can be built. It does provide acceptable oxine yields, produces minimal ^{68}Ge breakthrough, is simple enough for routine operation, and when the funnels are changed from glass to teflon, should produce gallium-68 pure enough for all of our potential investigations.

2. RADIOPHARMACEUTICAL PREPARATION

Liver Agent

As discussed in a previous Progress Report, we have investigated a liver agent, ^{68}Ga-ferric hydroxide colloid in animals. This preparation is an adaptation of the preparation of ^{113m}In ferric hydroxide colloid. The ^{68}Ga-oxine residue is dissolved in 50 µl of ethanol followed by the addition of $FeCl_3$, 1 ml of pH 7.5 phosphate buffer, and enough normal saline to produce 3 ml of solution. This colloid upon injection into rats, shows a distribution with greater than 80% of the activity in the liver and only a small amount of the activity remaining in the blood. We have studied this agent in a series of patients. Some of these images are shown in Figures 3-4. It should be noted that not only are conventional tomographic slices obtainable from these images, but it is possible to reconstruct the

data in both the coronal and the saggital planes enabling a better display of the data.

^{68}Ga-Labeled Platelets

In our prior work utilizing the separated ^{68}Ga from the EDTA generator and the 2-3 mCi obtainable from our prototype solvent extraction generator, platelet labeling efficiencies of approximately 50% or greater were obtained. As has been referred to above, when we prepared ^{68}Ga from the larger solvent extraction generator, the platelet labeling yield dropped to around 20% presumably due to the presence of metals produced radiolytically from the vessel containing the ^{68}Ge.

Preliminary studies utilizing the ^{68}Ga separated in teflon funnels leads us to believe that this approach can obviate this problem and that we will be able to prepare larger amounts of ^{68}Ga labeled platelets.

APPROACHES TO THE PRODUCTION OF ^{68}GA LIPOPHILIC COMPOUNDS

Since gallium ions show large absorbances in the ultraviolet (1), this analytical method was chosen to see if complex formation occurred with hexacyclen (the nitrogen analog of 18-crown-4). The free amine was prepared from hexacyclen trisulfate (Aldrich) by suspending the salt in 10 N $KClO_4$ and extracting the compound into chloroform. The ultraviolet scan showed a peak at 250 nm from hexacyclen which shifted to 248 nm upon addition of gallium trichloride. The gallium peak remained at 280 nm. When 8-hydroxyquinoline (oxine) was added to the solution, the characteristic wavelength of gallium oxine (260 nm) was visible, and the gallium hexacyclen peak disappeared. It was concluded from the above that gallium complexes with

hexacyclen but that its stability constant was less than that of gallium oxine. Perhaps the cavity of the azo crown is too large for the gallium cation to effectively interact with the binding sites.

Because the uv peak heights are not proportional to the species concentrations, other methods were investigated so that stability constants could be derived. A method for electrophoresis of group III metals (2) using Whatman paper and gel electrophoresis media as stationary phases and 0.05 M $NaNO_3$ as the mobile phase was applied. The voltage gradient across the 18 cm long strips was raised as high as 15 v/cm and various ^{67}Ga complexes were separated for twenty-four hours. The following separations were achieved, but the poor resolution (bandwidths of ± 1 cm) precluded the use of this system; Ga-EDTA (+ 5 cm), Ga-DTPA (0 cm), Ga-oxine (-3 cm), Ga-hexacyclen (-5 cm), and Ga-citrate (-5 cm).

High-pressure liquid chromatography was then used, anticipating more quantitative separations due to the large HETP values for available columns and the ease of detection using a NaI crystal and ^{67}Ga. In order to separate the complexes with ligands as lipophilic as crown and cryptands, reverse-phase chromatographic columns such as the corasil μ-bondapak C18 (Waters) were most likely to be successful. Initially, the gallium complexes were found to precipitate as the trihydroxide because separations required the use of water (plus isopropyl alcohol) which gave a large concentration advantage of hydroxide ion relative to ligand. This problem was countered by making the mobile phase 1.0 millimolar in the ligand, but the ^{67}Ga still precipitated onto the head of the column, probably due to the large number of silanol sites available on the packing support. Use

of porasil columns and paired ion chromatography (PIC^R reagents from Waters) lead to similarly negative results.

The method next utilized was polarography. Using a supporting electrolyte solution of 1 M NH_4Cl and 5 M NH_4OH (3), the reduction wave for gallium oxine was found to shift from -1.66 v to -1.82 v when 221 cryptand (221 Kryptofix from Merck) was added. The reductions occurred using Model 174A Polarographic Analyzer (Princeton Applied Research) equipped with a hanging mercury drop electrode, a platinum working electrode, a saturated calomel reference electrode and a dc scan rate of 50 mv/sec. When oxine was added to the gallium-221 cryptate, there was no shift of the reduction wave back to the potential of gallium oxine. In addition, cyclic voltammetry on the gallium-221 cryptate shows an increase in the oxidation potential by +3.8 mv, evidence that it is probably Ga^{+1} which is complexed by the cryptand (as expected from the radius-cavity ratio). Results were also favorable when the smaller cryptand Kryptofix 211 (Merck) was added to gallium oxine, although there was no appearance of a new peak, only disappearance of the latter. The anticipated gallium-211 cryptate reduction peak was probably shifted to a potential more negative than the breakdown potential.

Since the direct quantitation of stability constants requires reversible electrode reactions, various polarographic media were investigated (see Appendix of reference 4). The results did not show reversible gallium reduction, which may be explained by molecular orbital arguments. Because electrode processes obey the Franck-Condon principle, the lowest unoccupied molecular orbital (LUMO) of the reduced species must be close in energy to

the highest occupied molecular orbital (HOMO) of the electrode (5). Reversibly-reduced species have a LUMO closely matched to the HOMO of the electrode, so no reorganization of the species is necessary. Irreversibly-reduced species must first be perturbed to a transition state, however. This transition state is expressed as a linear combination of the ground and excited states of the complex. The higher the complex excited states, the greater the energy of the transition state (6,7). For six-coordinate gallium, the linear combination of the 4s, 4p and 3d atomic orbitals with the six ligand σ orbitals results in the fifteen molecular orbitals of Figure 5. The bonding MO's (t_{1u}, a_{1g}, and e_g) are filled by the twelve ligand electrons leaving non-bonding and antibonding t_{2g} and e_g^* for the ten bonding electrons of Ga^{3+} (electronic structure $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10}$). An environmental perturbation could split the t_{2g} or e_g^* levels by introducing asymmetry to the ligand field and thereby cause a smaller energy difference between them. However, since these MO's are completely filled it would be inconsequential to the reversibility of the process. That is to say, the LUMO is the antibonding a_{1g}^* which is difficult to perturb without altering the native metal-ligand interaction drastically. For this reason, the reorganization energy necessary for gallium complexes to match their LUMO to the mercury HOMO must be delivered by the electrode field, hence irreversibility results.

An alternative approach to gain gallium complex reversibility is to adjust the HOMO of the mercury electrode to the unaltered LUMO energy of the reduced species. This is apparently accomplished by performing the electrode reaction in high ionic strength polarographic media containing

redox catalyst sodium thiocyanate (8). The half-wave for Ga^{3+} in such a solution (5.9 M $KClO_4$, 0.1 M NaSCn, 0.1 M p-toluenesulfonic acid, pH 1) using a DME and SCE reference electrode is shown in Figure 6. The corresponding reduction wave for the same solution under differential pulse mode is seen in Figure 7. The reversibility of this process is evident in the linear plot of E_{dc} versus $\log(\frac{i}{i_d-i})$ in Figure 8 . Correction for i R drop of the chart recorder was unnecessary because a high-impedance circuit was used. Regression analysis (least squares fit) was applied to the points of this function corresponding to $|\log(\frac{i}{i_d-i})| \leq 1.5$, so that currents in the wave of Figure 5 from 3% above baseline to 97% of the diffusion current were accounted for. The result was a slope of 0.0227 v, which agrees well with the theoretical Nernstian value of 0.020 v for a reversible three-electron reduction. In addition, the intercept of -0.753 v versus SCE gives an accurate value for the half-wave potential. Efforts are presently underway to study the effects of ligands upon this reversible gallium reduction wave.

TIME ALLOTMENT

	Current % Effort	% Effort for Rest of Period
Michael J. Welch, Ph.D.	10	10
Barry A. Siegel, M.D.	10	10
Michel M. Ter-Pogossian, Ph.D.	5	5

Gary J. Ehrhardt, Ph.D. - was supported 50% off this project, he resigned to take a position at the University of Missouri, Columbia, MO. 2/28/79

Sivarajan Pandian, Ph.D. - Dr. Pandian will replace Dr. Ehrhardt; his anticipated starting date is June 18,1979.

EQUIPMENT

Milli R/Q water purifier: value - $895.00

PUBLICATIONS RESULTING FROM CONTRACT

1. Ehrhardt, G.J., Welch, M.J.: A new germanium-68/gallium-68 generator. J. Nucl. Med. 19:925-929, 1978.
2. Wagner, S.J., Welch, M.J.: Gallium-68 labeling of albumin and albumin microspheres, J. Nucl. Med. 20:428-433, 1979.
3. Ehrhardt, G.J., Wagner, S.J., Welch, M.J.: Applications of gallium-68 produced by the gallium-68-oxine generator. Second International Symposium on Radiopharmaceutical Chemistry, J. Labelled Compounds & Radiopharm. 16:111, 1979.
4. Welch, M.J., Tewson, T.J.: Radiopharmaceuticals for neurological studies. Presented at the Second International Symposium on Radiopharmaceuticals, Seattle, Washington, April, 1979, to be published by the Society of Nuclear Medicine.

5. Ehrhardt, G.J., Hood, R., Djordjevic, L., Welch, M.J.: The design and testing of an automated $^{68}Ge/^{68}Ga$ solvent extraction generator. To be presented at the 26th Annual Meeting of the Society of Nuclear Medicine, June, 1979, J. Nucl. Med. 20:682, 1979.

6. Welch, M.J.: Radiolabeled compounds of biomedical interest containing nuclides of gallium and indium. To be presented at the American Chemical Society meeting, Washington, D.C., September, 1979.

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1. Brown DM and Dainton FS, Trans Faraday Soc 62:1139, 1966.

2. Bailey RA and Steger A, J Chromatog 25:442, 1966.

3. Mika K, Chem Listy 50:43, 1956.

4. Meites L, Polarographic Techniques, Interscience, New York, 1965.

5. Vlcek AA, Disc Faraday Soc 26:164, 1958.

6. Vlcek AA, Coll Czech Chem Comm 24:181, 1959.

7. Hush NS, Z Elektrochem 61:734, 1957.

8. Moorhead ED, Frame GM, Anal Chem 40:280, 1968.

Table I

PERFORMANCE OF $^{68}GE/^{68}GA$-OXINE AUTOMATED SOLVENT EXTRACTION GENERATOR

	With Backwash	Without Backwash
^{68}Ga-oxine yield	55%	67%
^{68}Ge breakthrough	.001%	.004%

FIGURE CAPTIONS

FIGURE 1: A. Schematic of automatic solvent extraction generator

B. Photograph of generator with front shielding removed

FIGURE 2: Method of control of automatic generator.

FIGURE 3: ^{68}Ga iron colloid - PETT. Fifty-seven year old male with undifferentiated adenocarcinoma (unknown primary) metastatic to lymph nodes and bone. ^{99m}Tc sulfur colloid liver scan demonstrates multiple space occupying lesions in both lobes of the liver. Findings are confirmed on the CT scan. PETT scan demonstrates space occupying lesions at the junction of the right and left lobes, in the right lobe and in the left lobe seen best in slices 1 and 3 respectively.

FIGURE 4: PETT coronal reconstruction images closely parallel the anterior ^{99m}Tc sulfur colloid liver image. The levels at which the coronal sections were reconstructed are shown on the transverse PETT images.

FIGURE 5: Molecular orbital diagram for octahedral gallium complexation involving no π-bonding.

FIGURE 6: Reduction of gallium (III) perchlorate.

FIGURE 7: Reduction of gallium (III) perchlorate.

FIGURE 8: E versus $\log(\frac{i}{i_d - i})$ for wave in Figure 6.

AUTOMATED SOLVENT EXTRACTOR

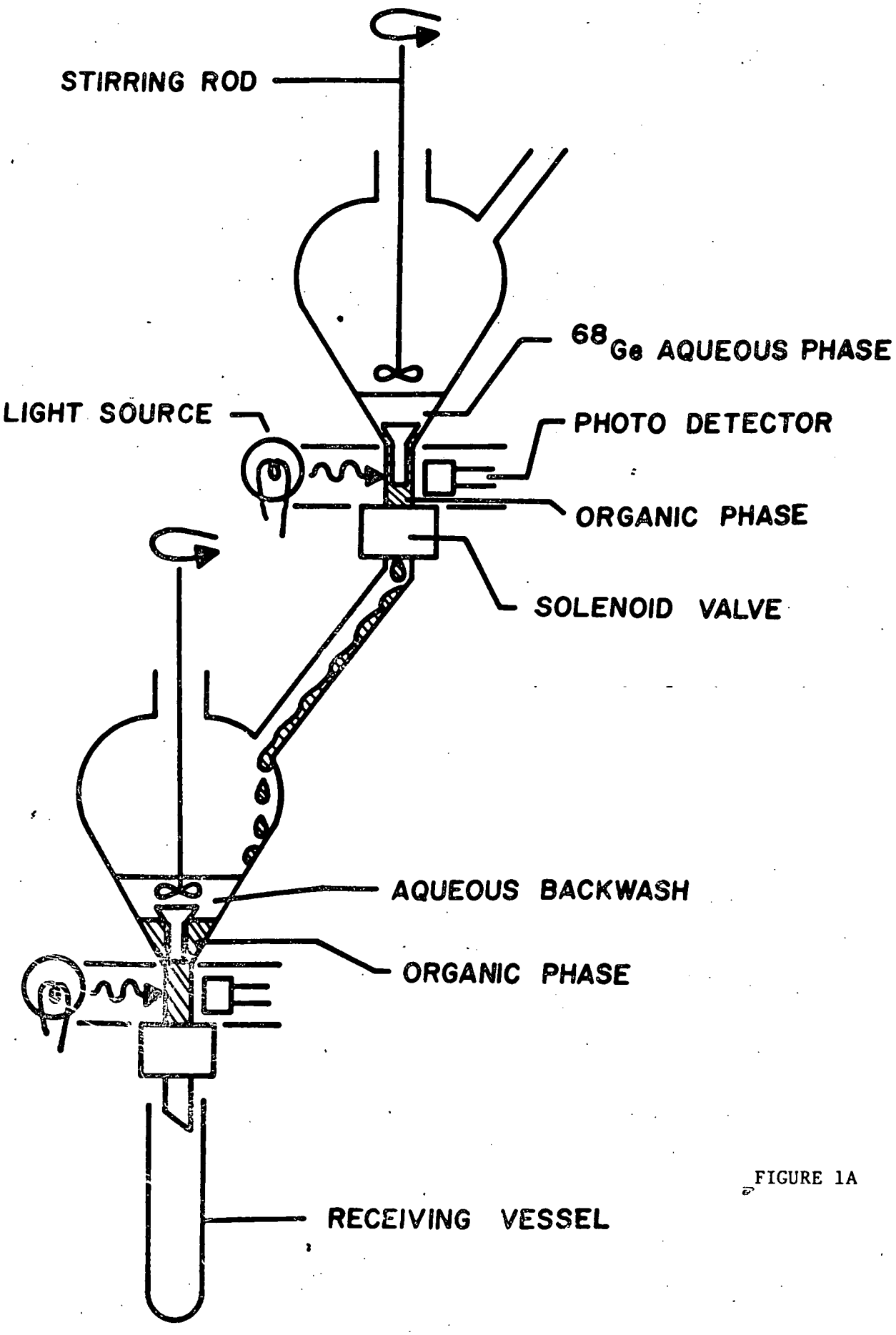


FIGURE 1A

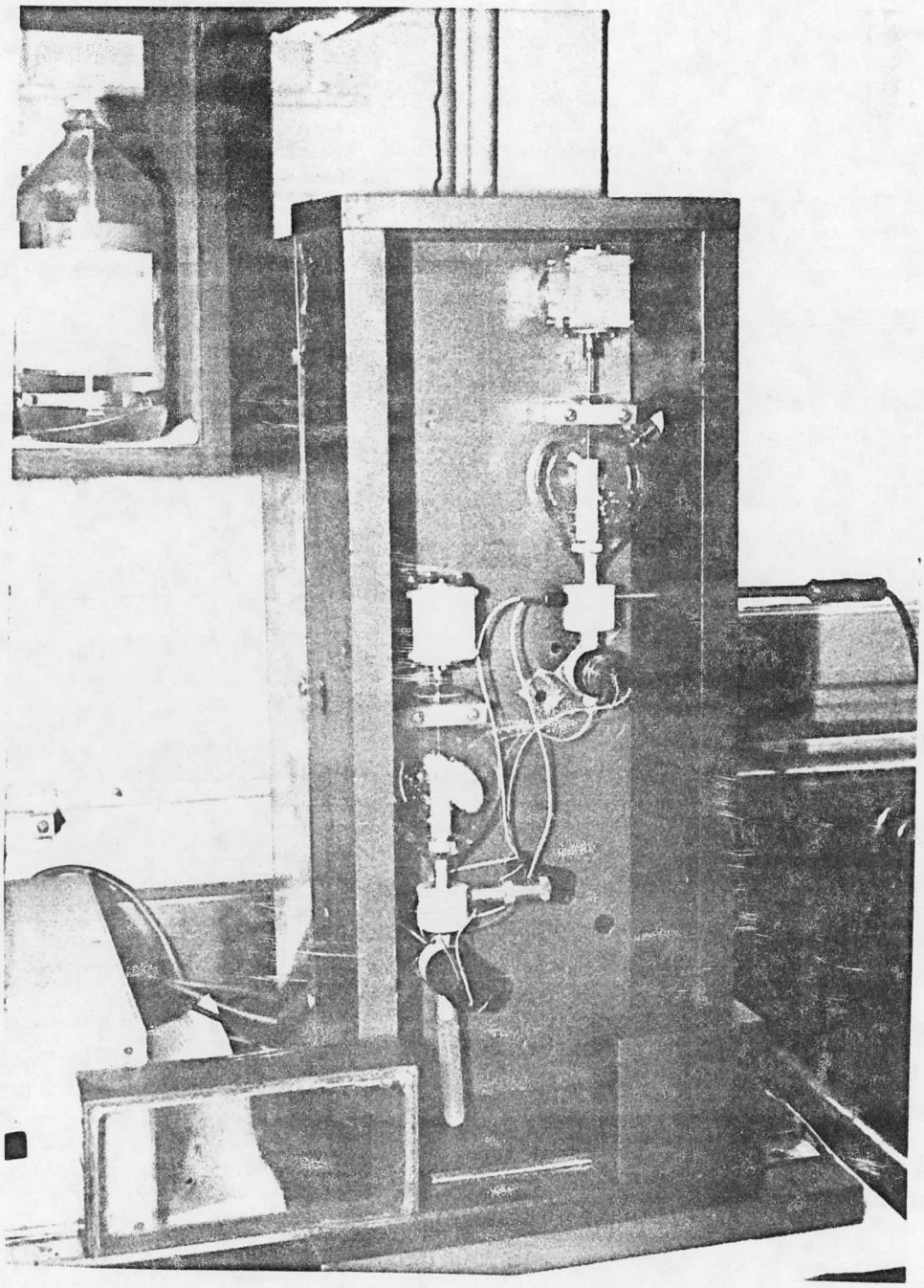
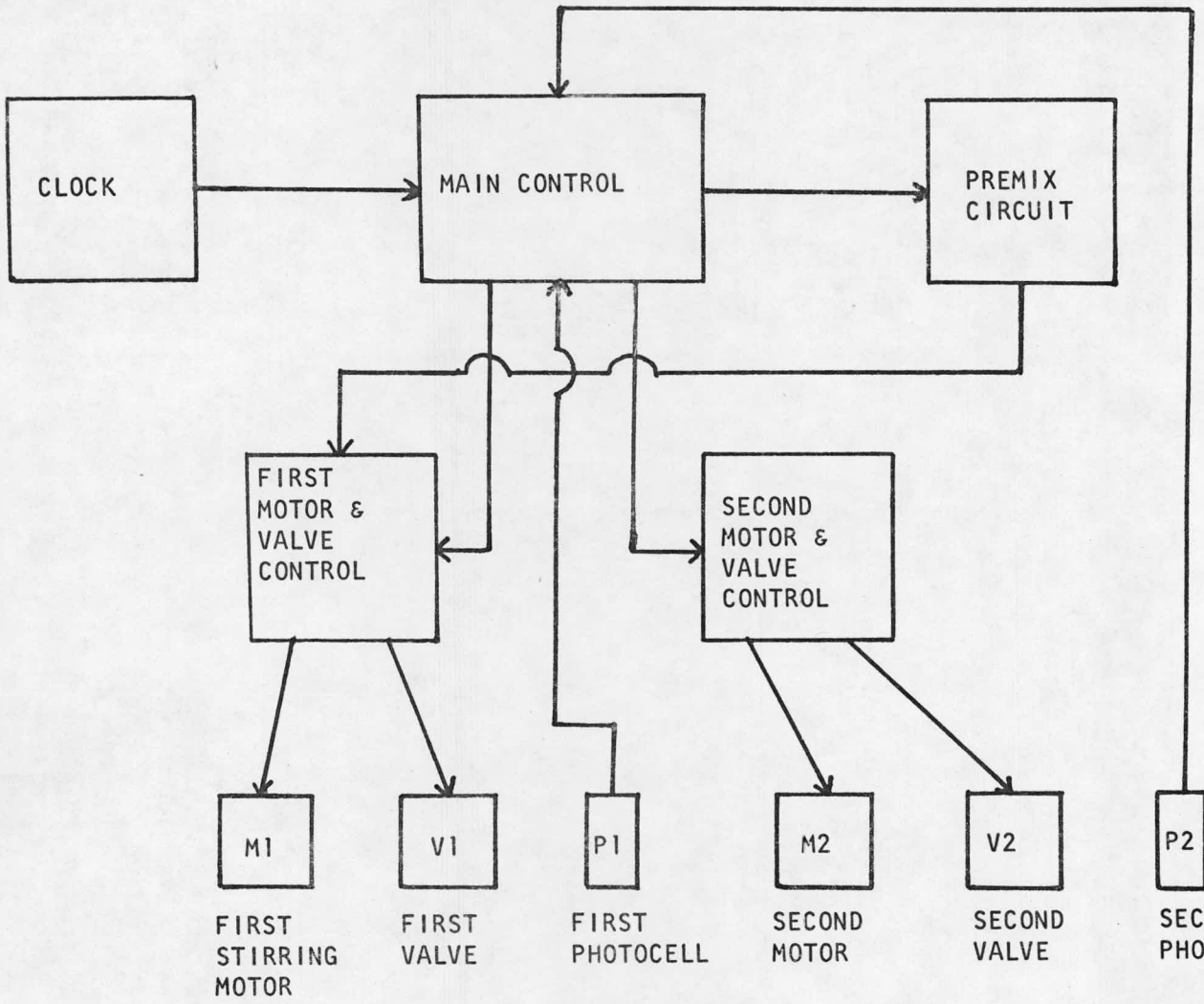


FIGURE 1B

AUTOMATED SOLVENT EXTRACTOR

CONTROL DIAGRAM



SEQUENCE OF OPERATIONS

PREMIX	0.5 mi
FIRST STIRRING	6 mi
FIRST PHASE SEPARATION	1 mi
DRAINAGE	0.5 mi
SECOND STIRRING	2 mi
SECOND PHASE SEPARATION	1 mi

FIGURE 2

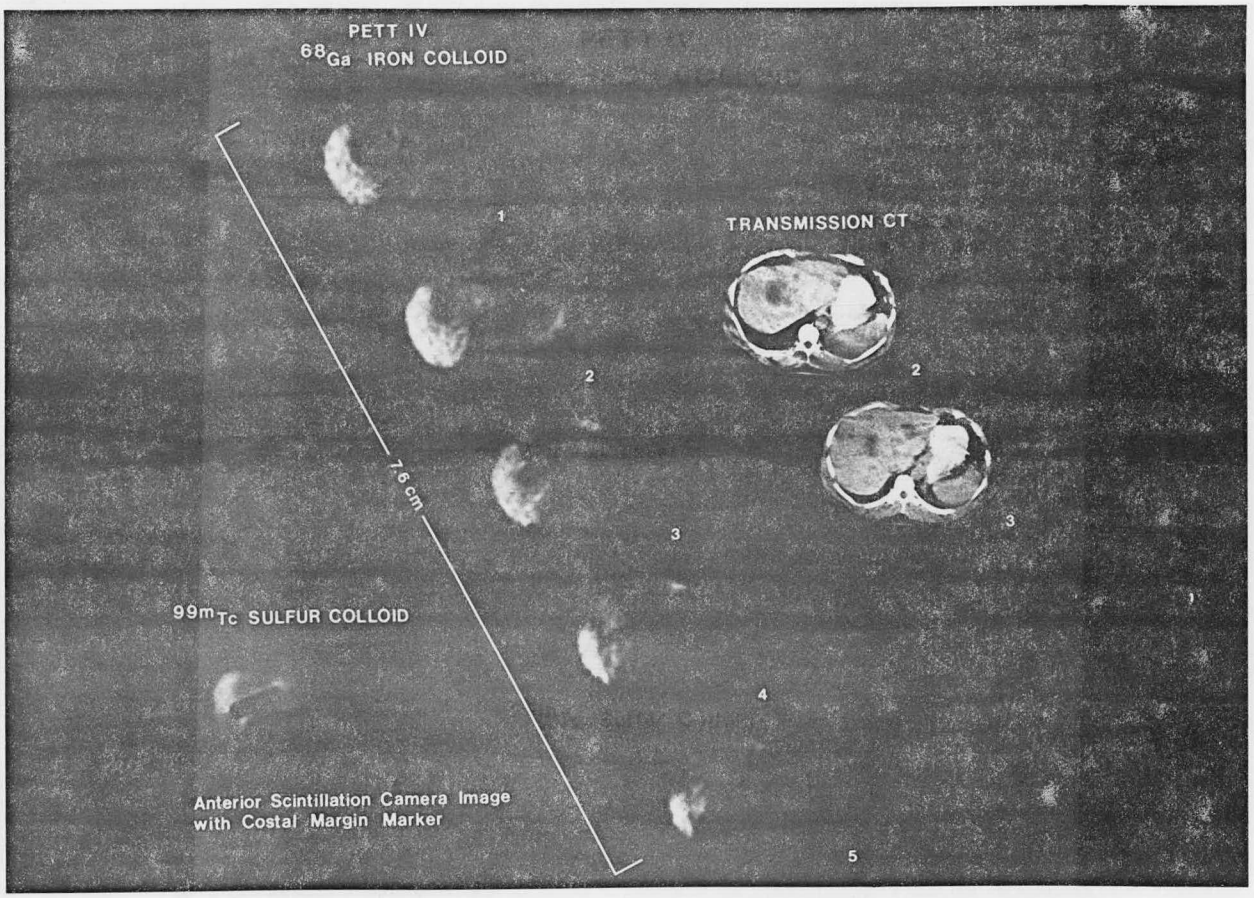

PETT IV
68Ga IRON COLLOID
TRANSMISSION CT
1
2
2
3
3
7.6 cm
99mTc SULFUR COLLOID
4
Anterior Scintillation Camera Image
with Costal Margin Marker
5

FIGURE 3

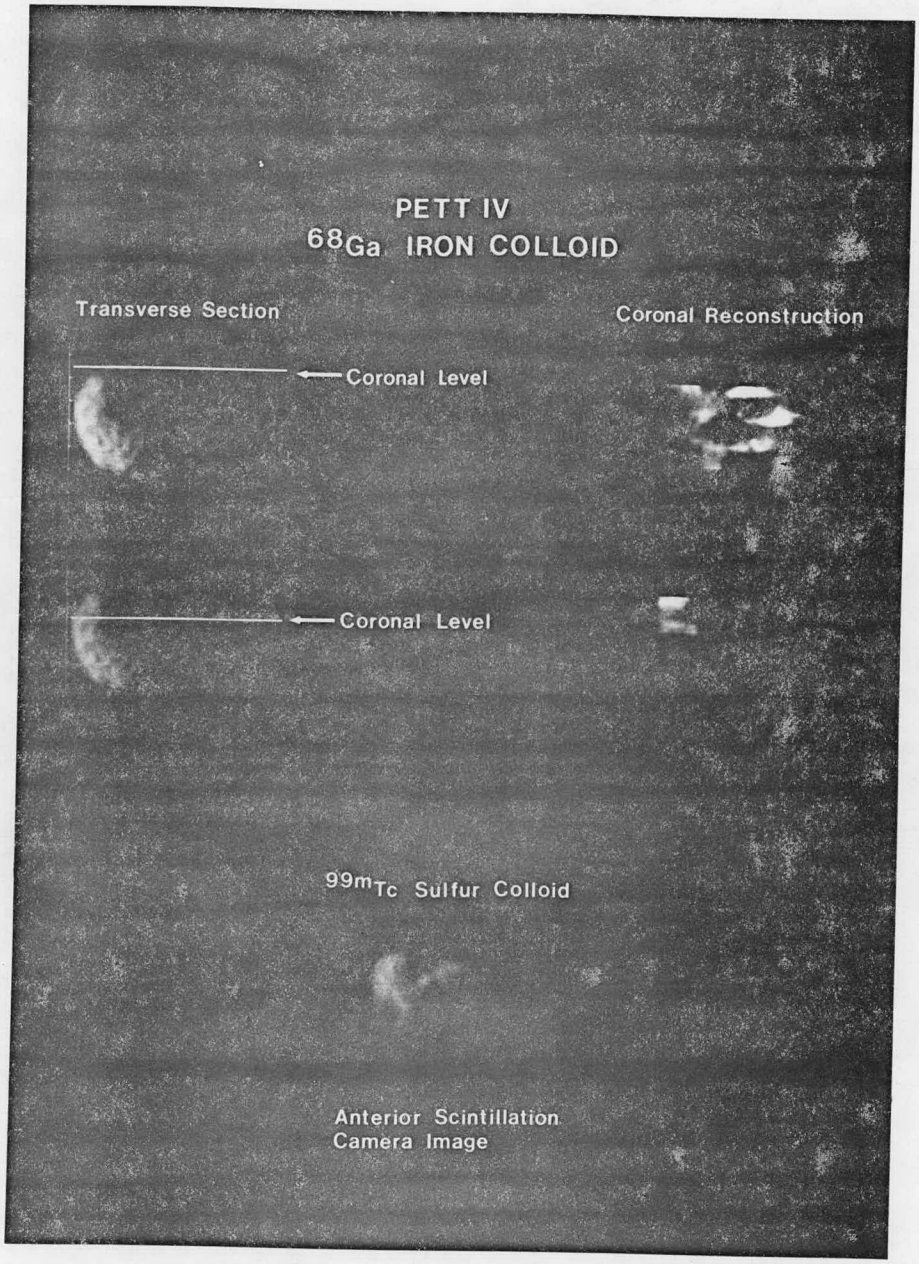

PETT IV
68Ga IRON COLLOID
Transverse Section
Coronal Reconstruction
Coronal Level
Coronal Level
99mTc Sulfur Colloid
Anterior Scintillation
Camera Image

FIGURE 4

FIGURE 5: MOLECULAR ORBITAL DIAGRAM FOR OCTAHEDRAL GALLIUM COMPLEXATION INVOLVING NO π-BONDING

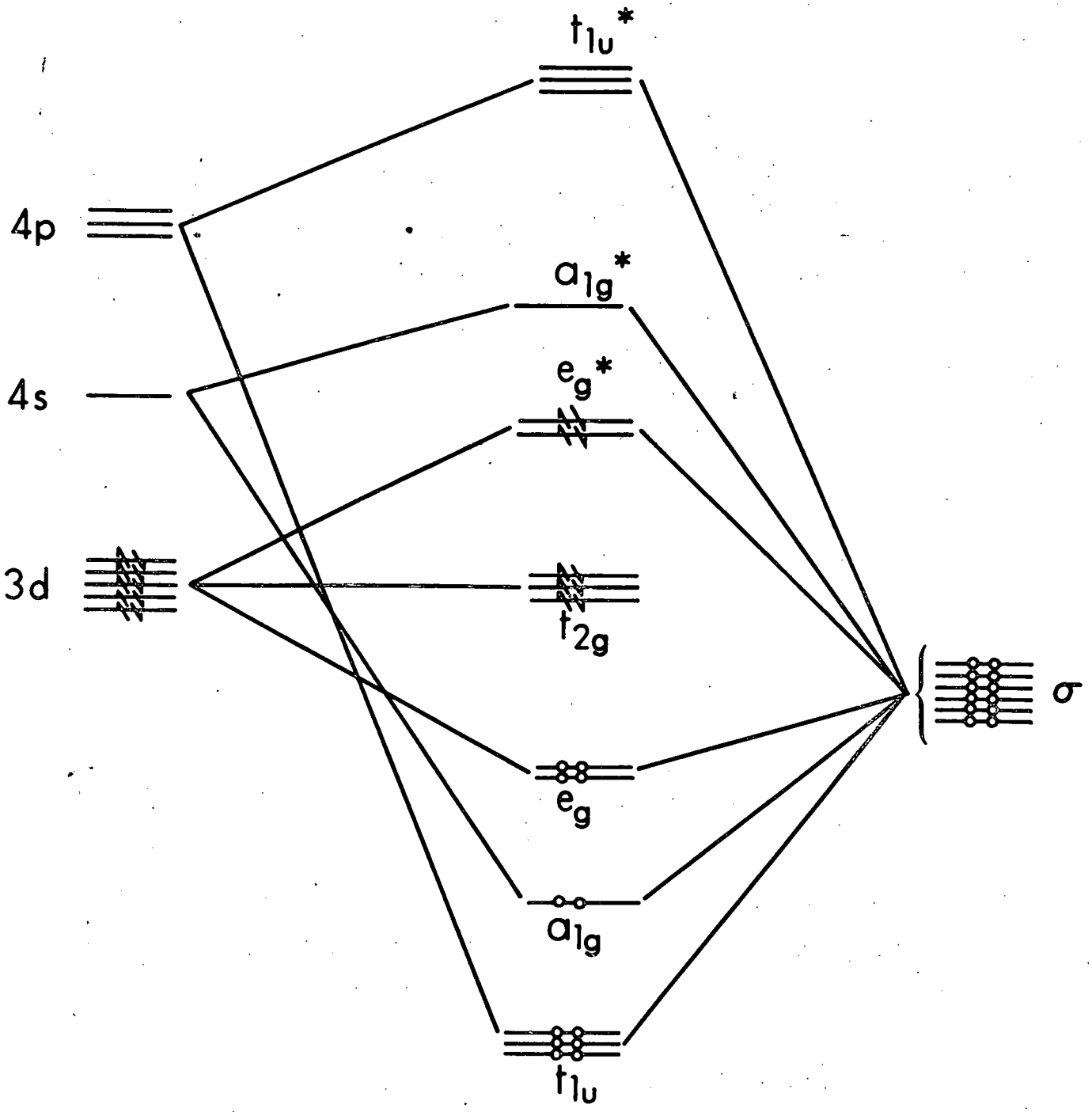


FIGURE 6: REDUCTION OF GALLIUM (III) PERCHLORATE

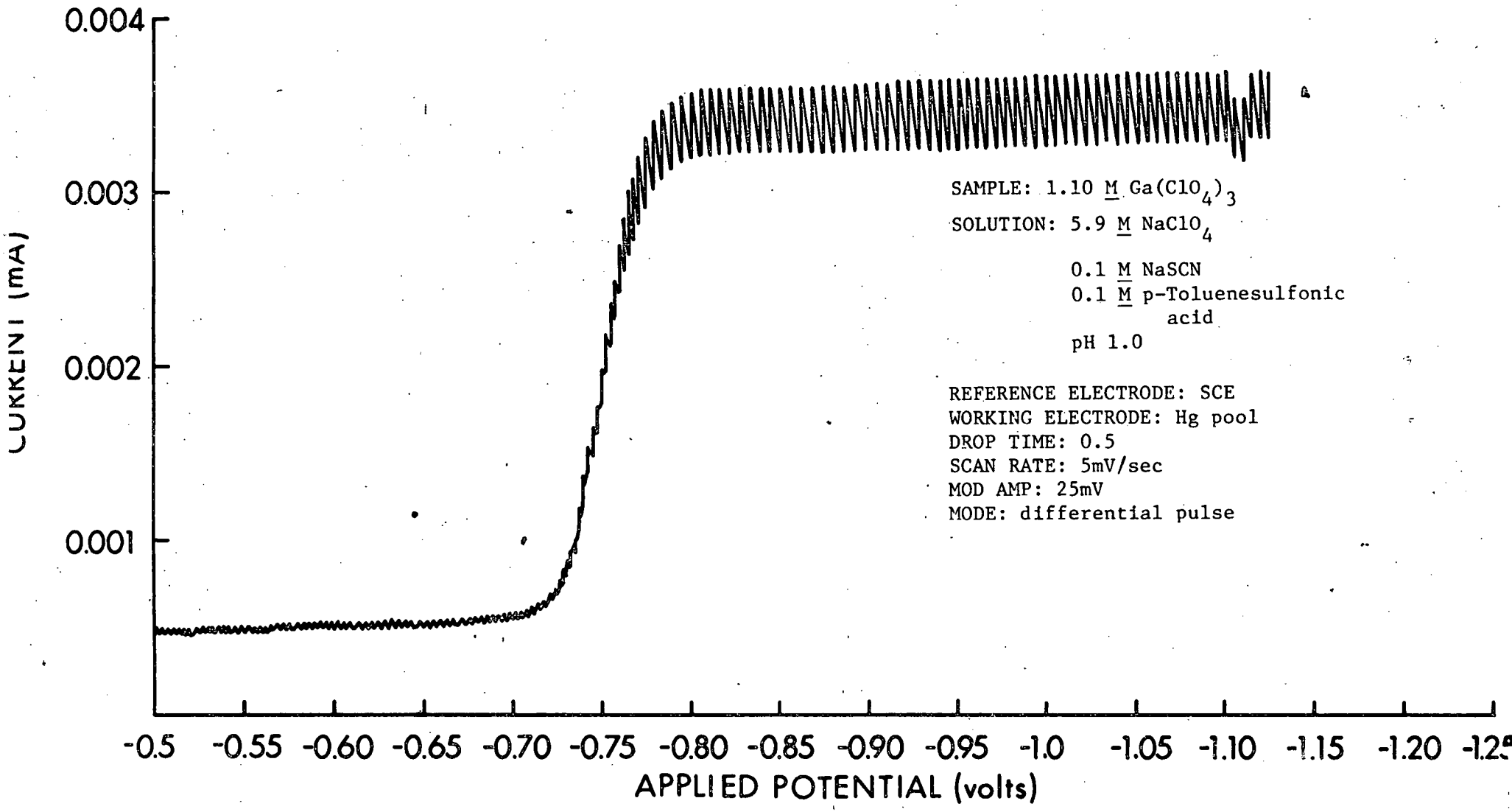


FIGURE 7: REDUCTION OF GALLIUM (III) PERCHLORATE

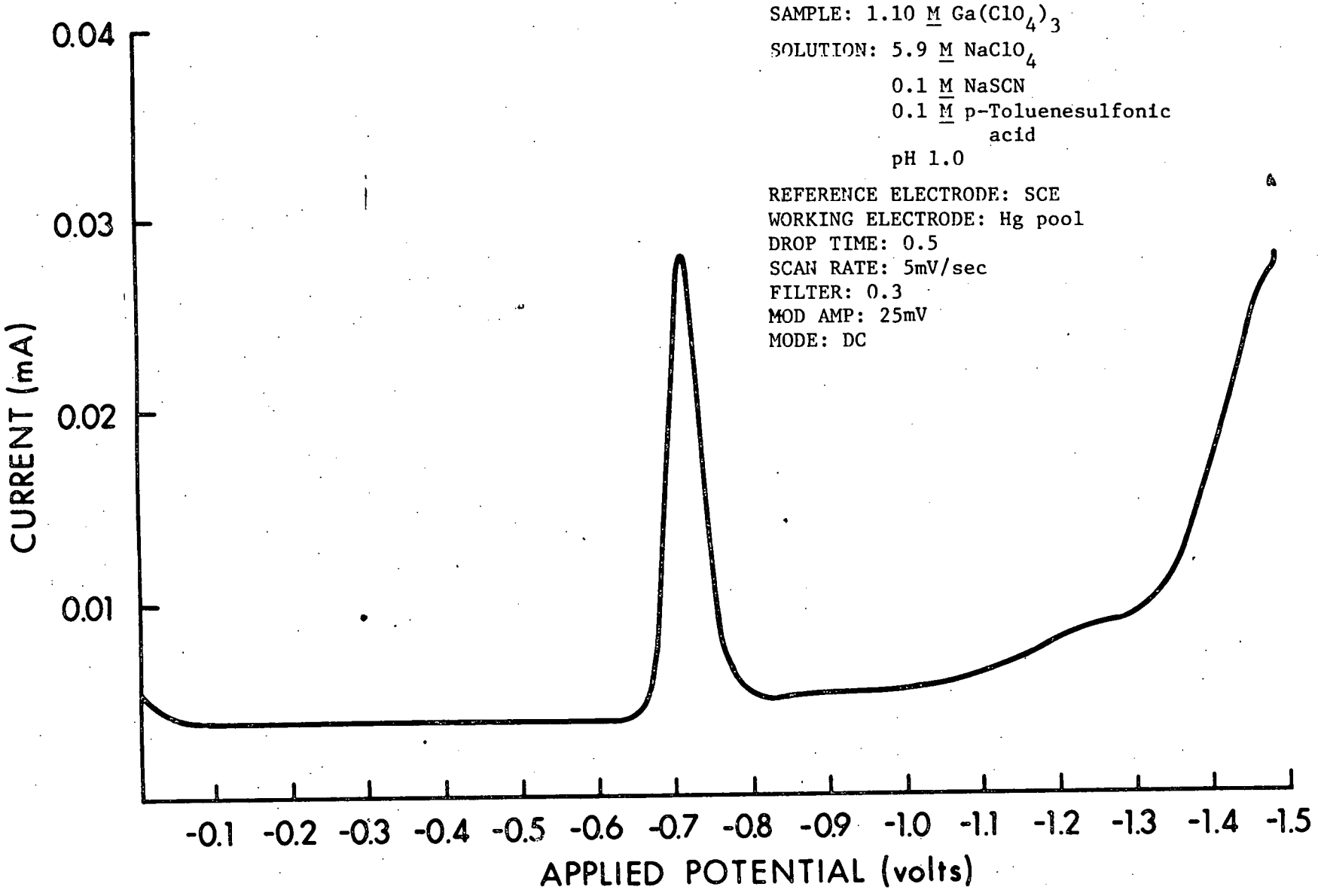


FIGURE 8: E VERSUS LOG $\left(\frac{i}{i_d - i}\right)$ FOR WAVE IN FIGURE 6