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A PROTEIN COATED PIEZOELECTRIC CRYSTAL DETECTOR

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13. ABSTRACT (Maximum 200 words)

The purpose of this project was to develop a protein coated, portable piezoelectric crystal detector for organophosphorus compounds. The performance of acetylcholinesterase, GD-1 anti-soman, anti-DMMP antibody, and bovine serum albumin (BSA) coatings was evaluated. Different immobilization methods were also tested. The responses obtained with the protein coatings immobilized via cross-linking with glutaraldehyde were acceptable, provided that the reference crystal was coated with dextran. The proposed coatings showed good stability and reasonable lifetimes that ranged from approximately three weeks in the case of the antibody coatings to several months in the case of BSA. Although moisture, gasoline, and sulfur are potential interferents, their effects on the sensor were eliminated by using a sodium sulfate scrubber which did not affect the performance of the detector towards organophosphates. A small, battery operated portable instrument capable of real time measurements with alarm function was produced. The instrument can be used in a wide range of applications, depending on the coating applied to the crystals.

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SUMMARY

A research study was conducted to develop a prototype piezoelectric crystal detector for organophosphorus compounds utilizing biocoating materials. This study showed that acetylcholinesterase, GD-1 anti-soman, anti-DMMP antibody and bovine serum albumin (BSA) can be successfully used if they are immobilized by the glutaraldehyde crosslinking technique and their responses are measured versus a dextran coated reference crystal. The sensors showed good sensitivities and reproducibilities at very low concentration ranges of organophosphorus simulants.

A prototype instrument was produced that meets the criteria outlined in the phase II proposal. The instrument is microprocessor controlled and features a dual crystal chamber, frequency counter, air pump, digital display, RS-232 output and alarm. In addition, sensors for temperature and humidity are included.

The technology developed in this project could have many applications in industrial hygiene monitoring in both civilian and military sectors. The instrument can be used for monitoring exposure to different pollutants if the proper coating is applied on the sensing crystal.

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PREFACE

The work described in this report was authorized under Contract No. DAAA15-87-C-0004. This work was started in March 1987 and completed in August 1989.

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1. INTRODUCTION

The field of biosensor technology is one of the most expansive areas in biotechnology with a seemingly limitless potential for growth. The number of publications and journals dedicated specifically to this area and the number of internationally organized conferences on the subject are testament to this fact. In a recent CRDEC meeting in Aberdeen, Maryland, greater than 60% of the papers and posters dealt with the use of biologically active material (enzymes, antibodies, microorganisms, etc.) for detection and/or decontamination purposes.

With the current state of development and technology in the area of biotechnology, the use of chemical warfare agents by an enemy in battle is no longer just a probability, but a very likely possibility. Organophosphorus agents and other cholinesterase inhibitors are the most likely agents because of their potency and speed of action. Fortunately, it is possible with the right biosensors to detect these agents in time to remove personnel from areas where such agents have been used, and/or to decontaminate those areas with large amounts of these agents using their antibodies, antidotes or large amounts of water.

A group specific sensor as well as an agent specific biosensor should be of great interest to the defense department (Army, Navy, Air Force, etc.). The group specific biosensor can be used by personnel in a battle field situation to alert them to

the presence of a particular class or group of chemical warfare agents without necessarily identifying a particular agent. This early warning type device would give the troops enough time to escape any further danger and exposure. The agent specific detectors can then be used to specifically identify and quantify the agent.

It is indeed possible to develop both biosensors using current technology. Acetylcholinesterase catalyzes the hydrolysis of acetylcholine to acetate and choline. This reaction is fast, going to completion in about 40 microseconds. This reaction is crucial for the rapid repolarization of the postsynaptic membrane essential for nerve impulse transmission. Without this repolarization, death comes swiftly through respiratory paralysis and asphyxiation. In this reaction acetylcholine reacts with the serine residue in acetylcholinesterase, forming an enzyme-acetylcholinesterase (Enz-ACh) complex, with the release of choline. The Enz-ACh complex breaks down very rapidly in the presence of water to regenerate the enzyme and acetylcholine, which continues the cyclic process.

Organophosphate agents and other acetylcholinesterase inhibitors form a covalent intermediate with the serine residue on the enzyme in a process similar to that formed by the enzyme substrate, acetylcholine, but this enzyme-inhibitor complex is much more stable and breaks down in the presence of water at a rate much slower than that of the Enz-ACh complex. This

effectively blocks enzyme activity including synaptic impulse transmission in mammals, leading to asphyxiation and death from respiratory paralysis in a very short time.

Acetylcholinesterase can be used, then, as a basis for the development of a group specific type biosensor for organophosphate agents and similar acetylcholinesterase inhibitors. The piezoelectric crystal detector using acetylcholinesterase coated crystals is one approach that we think could be used to develop a group specific biosensor. Also, the use of agent specific antibody coated crystals is a feasible approach to developing agent specific biosensors.

2. OBJECTIVES

The objectives of phase II were: 1) the development of optimum "protein coatings useful for the detection of organophosphates and nerve agents", 2) the evaluation of these coatings, and 3) the development of a "portable microprocessor based instrument" capable of driving the sensors developed, and reading the resulting frequencies, and converting the frequencies to a real time concentration readout.

3. EXPERIMENTAL

I. MATERIALS:

Acetylcholinesterase (electric eel, Type V-S), gelatin, (calf skin, #G-9382, 225 Bloom), dextran, DEAE dextran, glutaraldehyde grade II and bovine serum albumin (BSA, #A-7906)

were purchased from Sigma Chemical Co.

Diisopropylmethyl phosphonate (DIMP) and dimethyl methyl phosphonate (DMMP) were obtained from Alfa Chemical Co. and Aldrich Chemical Co., respectively.

Dulbecco's buffer, a phosphate buffered saline solution, was composed of 137.0 mM NaCl, 2.7 mM KCl, 8.0 mM $Na_2HPO_4.7H_2O$, and 1.5 mM KH_2PO_4.

GD-1 (IgM) anti-soman, monoclonal antibody was produced by the Southwest Foundation for Biomedical Research and sent to us on instructions from Col. Jerry Sadoff, of The Walter Reed Army Research Institute (Washington D.C.). The antibody was produced in 1984, in Georgetown using a homologue of soman (moiety) conjugated to KLH as the immunogen. The antibody was purified using a goat, anti-mouse IgM column, and showed no KLH antibody activity.

Rabbit, anti-DMMP antibody was produced by Berkeley Antibody Company using a DMMP-KLH conjugate on a sub-contractual basis for this project. The immunogen was a conjugate of DMMP to BSA via the lysine residue of the protein. DMMP-KLH was prepared in a similar manner using the acid derivative of DMMP. Purification was carried out using DMMP-BSA affinity column.

A gas capillary tube was obtained from VICI Metronics. Permanent gas permeation tubes for DIMP and DMMP were purchased from Kin-Tek Laboratories and G.C. Industries respectively.

II. APPARATUS

A schematic of the instrumental set up is shown in Figure 1. The instrumentation consisted of a low frequency mode OX transistor oscillator (International Crystal Co.) powered by a regulated power supply model IP-28 (Heath Zenith Electronics). The frequency output from the oscillator was measured by a digital frequency counter (Heath Zenith Electronics), modified by a digital-to-analog converter so that changes in the frequency of the crystal could be recorded. The piezoelectric crystals used were gold coated, 10 MHz, AT-Cut, quartz crystals (Bliley, Erie, PA). Calibrated and bubbler flowmeters were purchased from Suppelco and Alltech Associates, respectively. Temperature monitoring was made with a digital multimeter (Radio Shack), and the relative humidity was monitored with a digital hygrometer (model HI8064, Cole Parmer Ind. Co., Chicago, IL). A Lauda MS-3 temperature controlled circulating water bath was purchased from Baxter Scientific.

Dry grade compressed air, from Linde Specialty Gases, was passed through a one meter tube containing Drierite, then split into two streams, A and B.

Stream A flowed through a flow meter and a humidifier and was combined with the resultant air stream from stream B at a three port (T) valve before the total air stream flow meter, F. The humidity level was varied by means of the humid air flow meter.

Gas stream B was split into two streams B_1 and B_2. B_1 was divided into four streams which flow through flow meters 1, 2, 3

and 4 and their respective sources for DMMP, DIMP, and interferants (5, 6, 7, and 8 respectively). Between 5 and 6, 7 and 8 there were two four-port valves for selecting two gases. Another four-port valve joins these two streams to select one sample gas to be measured. B_2 was split into two streams, C and D, which were the diluent and background gas streams.

A four-port valve was placed between the sample stream and the background stream so that the chamber could be purged with pure background gas between measurements. The resultant gas flows through an injection port to the PZ-105 detector.

4. METHODS

I. COATING PROCEDURE:

The same general coating procedure was used for all crystals. In a few cases, the crystals were washed with surfactant and rinsed with buffer or water before protein coatings were applied. Prior to coating, the base frequencies of all crystals were first determined by placing each crystal in the crystal chamber and compressed dry air or compressed dry nitrogen was allowed to flow past the crystal at a flow rate of either 50 or 100 mL/min.

A solution of either gelatin or bovine serum albumin (BSA) and enzyme, acetylcholinesterase, was prepared in 0.1 M phosphate buffer, pH 7.0-7.4. Less than two microliters of this mixture was applied to the electrode on one side of the crystal with a microliter syringe, and spread over the electrode surface evenly.

A volume of glutaraldehyde corresponding to approximately ten percent of the volume of the BSA/enzyme or gelatin/enzyme mixture was then added, mixed well and allowed to dry.. The other side of the crystal was then coated in a similar fashion. Care was taken during the coating procedure to insure an even film on the electrode surface. After drying, the crystals were washed with buffer (0.1 M in phosphate and 0.1 M in glycine, pH 7.0) to inactivate all unreacted glutaraldehyde, followed by 0.1 M phosphate buffer, pH 7.0, and then, in some cases, finally with Dulbecco's buffer and deionized water. In some instances, a thin coating of sodium lauryl sulfate was applied to the electrodes before the immobilization procedure. The entire wash procedure was eventually eliminated due to poor precision in the amount of coated mass. Crystals were stored in a desiccator when not in use.

The amount of protein in the antibody preparation was sufficient; therefore, no BSA was used on most of the active crystals. BSA also was frequently used on the reference crystals with or without glutaraldehyde, depending on the coating procedure. Reference crystals were subsequently coated with dextran.

II. MEASUREMENTS

All frequency measurements were made in a flowing stream of gas at a predetermined flow rate. Base frequency measurements of the uncoated crystal were made after a stable background was

established. After drying, the base frequencies of the coated crystals were remeasured to obtain a new base frequency shift as a result of the coating. In either of these two cases, only pure background gas was allowed to come in contact with the crystal.

For actual sample measurements, the coated crystals were allowed to come to a stable baseline in the pure background carrier gas. To make a measurement, a four-port valve was used to redirect the sample vapors to the crystal chamber. Changes in frequency were recorded every 30 seconds as the sample continuously flowed past the crystal, for at least 2 minutes, before switching back to the pure background carrier gas. To repeat a measurement, a 10 minute recovery period was needed for the crystal to return to the pre-sample introduction frequency and also to purge the system of any sample that might be in the crystal chamber.

At the beginning of each day, the system was purged with the vapors saturated with the sample, to minimize the absorption of sample by the different components of the generation system. Initially, a single crystal chamber made from glass was used. Then a new chamber made from polycarbonate (Lexan) was designed to hold two crystals and to provide the same degree of exposure to the substrate. Finally, the PZ-105 was incorporated in the set-up, and both frequency changes of the inactive and active crystals were made simultaneously.

5. RESULTS AND DISCUSSIONS

Both the enzyme and antibody coated crystals were evaluated in basically the same way. First, the amount of coating was determined to see if the same mass of coating produces the same magnitude of base frequency shift (KHz). All crystals that were vibrating were subsequently tested with their corresponding pairs for response to DMMP, DIMP, and interferences.

The linear range, stability, reproducibility, response time and lifetime of the coatings were evaluated.

I. ACETYLCHOLINESTERASE

Acetylcholinesterase of different activities, either in solubilized form or immobilized on the crystal, was tested. Several conventional and modified immobilization techniques were evaluated, including cross-linking agents, bovine serum albumin or gelatin/glutaraldehyde and wetting or surfactant agents: sodium lauryl sulfate, Tween 80, Triton X-100 and Tergitol.

a. Immobilization Technique

The use of wetting agents was proposed to achieve better reproducibility and ease of regeneration of the gold electrode for further use. Details of the immobilization solutions, crystal coatings and crystal response on a representative group of crystals are shown in Tables 1, 2 and 3, respectively. The BSA/ glutaraldehyde crosslinking technique was found to be the most sensitive (Table 4). Although the addition of wetting agents improved reproducibility slightly, it did not improve the response. Subsequent studies showed that the amount of

glutaraldehyde in the mixture should be approximately 5-10% (Tables 5 & 6).

Initial studies were performed using a bubbler to generate the desired concentration of DMMP. Additional experiments carried out with a permeation device for DMMP led to similar conclusions. However, the magnitude of the response using the bubbler was about six times of that using the permeation device. Also, the reproducibility with the bubbler was poorer.

b. Response vs. Amount of Enzyme

The forementioned studies were carried out using a blank (uncoated) reference crystal. The poor reproducibility was partially due to nonselective absorption or possibly the condensation of the analyte on the coating. The response of enzyme coated crystals was re-evaluated versus bovine serum albumin (BSA) coated reference crystals. As evident in Table 7, the magnitude of the response decreased significantly when the reference crystal was coated with BSA. Also, it seems that the total weight of the coated protein had a more pronounced effect on the response than the activity of the coated enzyme (see Table 8 and Figure 2).

Crystals #1 through 14R showed no definite response patterns to various concentrations of dimethyl methyl phosphonate (DMMP). The poor responses were the result of applying more mass to the reference crystals than the active crystals, using masses in excess of 100 μg and using ten percent glutaraldehyde in the coating procedure.

Ten percent glutaraldehyde contributed between twenty and seventy five percent of the total coating mass. It was believed that using concentrations of glutaraldehyde of above one percent could keep the enzyme from interacting with the substrate by tying up the binding sites of the enzyme. The optimum concentration of glutaraldehyde in the final mixture was found to be less than seven percent, with a one percent or lower initial concentration the best.

For a similar range of coatings, crystals #15 through 20R also produced unsatisfactory results. Most of the crystals, as noted in Table 3, went into overtones. This seemed to be due to overloading and/or an overpowering amount of glutaraldehyde. No reasonable results were obtained using this set of crystals.

Perhaps the most promising results were obtained using crystals #21 through 26. These crystals had less coating mass than most of the crystals mentioned above (in Table 2 and 3), but were coated using the same concentration of glutaraldehyde. The responses of crystals #21-22R were closer to theoretical because the coating mass of the reference crystals was less than the mass applied to the active crystals. Based on the amount of coating, in μg/crystal, the responses from the inactive crystals initially seemed to be consistently lower than those from the active crystals.

Figure 2 shows the responses of crystals #23-26 as a function of the amount of coatings in μg/crystal. The total mass of coating per crystal was the amount of material that was

actually applied to the crystal. Obviously, not all of this material was immobilized on the electrode. Most of the non-immobilized protein and salts were washed off in the rinsing process. Such a plot, therefore, does not truly reflect an accurate response but gives a qualitative indication of how much weight could be put on the crystals. The washing process was eliminated in the coating protocol to reduce such errors in precision in the coating process .

Figure 2 shows the response of both active and inactive crystal pairs with the "same" amount of coating per crystal. Both active and inactive crystals showed an increase in response with an increase in the amount of coating. The magnitude of response between each pair, however, seemed to remain constant with a corresponding increase in the amount of coating. This response profile gave an impression of nonselectivity.

Figures 3 shows responses as a function of the actual amount of coating available on each crystal, in KHz. Figure 3 also shows the response of the active and inactive crystals as a function of the base frequency shift, in KHz. The plots show a fairly good linear relationship between the response and the base frequency shifts. This was significant, because it confirms the existence of a linear relationship between the response and the amount of coating, it also indicates that this might be a nonspecific and/or nonselective process.

Figure 4 is a plot of the response as a function of the amount of enzyme units per crystal. This plot also contains

results from the corresponding reference crystal pairs. The corresponding reference crystal (#23R to 26R) had no enzyme immobilized on their surfaces. Responses from these crystals therefore represented the magnitude of the nonselective behavior of the coatings. The true responses, (active minus the inactive responses) as indicated by this plot, were small.

c. Calibration Curve

A typical calibration curve of response to DMMP is shown in Figure 5. The curve was curvilinear in the concentration range of 140-900 ppb. The 900 ppb concentration was the maximum that could be obtained by the permeation device. As indicated in Table 9, 90% of the response was obtained within 90 seconds of exposure time. While only about 50% of the response was obtained in 30 seconds, the signal was large enough to be useful for fast assay of the DMMP present. As little as 140 ppb can be detected.

INHIBITION STUDY

In order to determine if DMMP actually inhibited the enzyme activity in the gas phase, we decided to carry out a number of inhibition studies. Crystals were coated with acetylcholinesterase and exposed to DMMP in the gas phase for different lengths of time, then colorimetric enzyme activity assays were performed. The results for a representative group of crystals are shown in Table 10.

The results were inconclusive, and it was thought that crystals might not be ideal for such a study because of possible

contamination from crystal mounts. The enzyme was subsequently immobilized on Biodyne membranes, mounted on plastic electrode jackets by an O-ring.

The membranes were exposed to gaseous DMMP for the same length of time used for the crystal exposures as shown in Table 10. The results from this study were also inconclusive.

II BOVINE SERUM ALBUMIN (BSA)

Because of the fact that some BSA coated reference crystals exhibited higher frequency changes than the enzyme coated active crystals, and the obvious independence of response on the activity of the enzyme coating, we investigated the possibility of using BSA as a coating. Crystals were coated with exact amounts of protein (enzyme, enzyme/BSA mixture, BSA) and their response to DMMP was evaluated. This study confirmed our previous suspicion that the magnitude of the response was affected by the total amount of protein coating used, and not by the activity of the enzyme (Tables 11, 12, 13). BSA was used as a coating material versus a number of reference coatings (sucrose, gelatin, dextran, and various GC stationary phases). Coatings of BSA or BSA with glutaraldehyde consistently gave higher responses than any other reference coating used. The optimum amount of BSA and BSA/glutaraldehyde ratios were determined from Tables 12 and 13. The highest response was obtained when 0.5 μL BSA (4 mg/100 μL) in phosphate buffer and 0.5 μL glutaraldehyde (0.1%) were applied to each side of the

crystal (a BSA/glutaraldehyde ratio of more than 10:1). A typical calibration curve for a crystal using BSA as coating versus dextran reference is shown in Figure 6. As little as 15 ppb of DMMP can be detected and linearity extended to 120 ppb. The response time is two minutes.

III. GD-1, ANTI-SOMAN ANTIBODY

a. Immobilization Procedure

Antibody, bovine serum albumin (BSA) and gelatin solutions were prepared as shown in Table 14, by dissolving the appropriate amount of GD-1, BSA and gelatin in Dulbecco's buffer (a physiological buffer), pH 7.45. The mass of the antibody on the sensing crystal was equal to the mass of BSA on the reference crystals. The respective amounts were immobilized with the aid of glutaraldehyde (10%, v/v) on both side of the crystals. The crystals were subsequently allowed to dry in a closed desiccator. The details of the coating contents are summarized in Tables 14, 15 and 16.

b. Response vs. Amount of Coating (KHz)

Figures 7 shows the typical response patterns portrayed by most of the crystals to DMMP. This figure shows a linear relationship between the response and the amount of coating on the sensing (antibody coated) crystal. The corresponding reference crystals (BSA coated) showed very little or no response to DMMP (Figure 7) probably due to the high content of glutaraldehyde in the coating.

c. Rate of Response Curves

A typical response versus time curves is shown in Figure 8. Based on earlier results, we found out that 90% of the response was completed within the first 2 minutes. Consequently, crystals were exposed to substrate vapors only for 2 minutes. This aided in both the recovery time and the assay time. As this figure shows, the response was fast and reversible.

IV. ANTI-DMMP ANTIBODY

a. Immobilization Procedure

Anti-DMMP, bovine serum albumin (BSA) and gelatin solutions were prepared, as shown in Table 17 and 18, in Dulbecco's buffer (a physiological buffer), pH 7.45. The mass of the antibody on the sensing crystal was equal to the mass of BSA on the reference crystals. The respective amounts were immobilized with glutaraldehyde (10%, v/v) on both sides of the crystals. The crystals were subsequently allowed to dry in a closed desiccator.

b. Response vs. Amount of Coating

The response of the anti-DMMP antibody coated crystals are given in Table 21.

Figures 9 and 10 are representative curves for the responses of the anti-DMMP antibody coatings to 2.00 ppm DMMP as a function of the amount of coating in KHz and μg respectively. There is a correlation between the amount of coating or the total mass (μg) and the frequency change (KHz). However for some crystal sets, the response to 2.00 ppm DMMP did not correlate with the base

frequency shifts (Figure 11). This phenomenon could be due to a non-uniform coating on the crystal surface. The response stayed constant due to saturation on the first layer.

c. Calibration

A calibration curve was constructed using crystals #178 and #174R as shown in Figure 12. It is apparent from the graph that the responses for the antibody coated crystal were linear in the concentration range of 0 to 2.00 ppm. A standard deviation of 1.73% at 0.35 ppm and 5.2% at 2.00 ppm at room temperature was obtained, by repeated assay of two coated crystals to DMMP.

d. Rate of Response

Figure 13 shows typical response curves and the relative rates of response for the anti-soman antibody coated crystals. The actual rate of decrease in frequency of the active and reference crystals as a function of time shows quite clearly the magnitude of the frequency difference between the two crystals.

V. TEMPERATURE

In the temperature study, the carrier gas was passed through a coil placed in a controlled thermostated bath and the temperature of the bath was varied to attain the desired temperature in the chamber. The response of the enzyme and BSA coatings decreased by 54-75% in the temperature range of 35-45°C indicating that the adsorption of DMMP on the surface of the coating at high temperatures was not favorable.

Two antibody coated crystals were studied for the effect of

temperature on their response. Temperature was measured by the temperature sensor embedded in the base of the crystal chamber. The crystals responses were measured at a specified temperature for an average of five measurements.

For the antibody coated crystals, the response also decreased with the temperature increase, as shown in Figure 14. Over the 38°C increase in the temperature range of 24-62.8°C, there was a decrease in response of 72-88%. Although 62.8°C may seem to be an excessive temperature, this temperature could be reached in an enclosed area, in a hot environment. After evaluating the crystals at 62.8°C, the system was returned to ambient temperature. The responses were examined again at 24°C and the crystals responded within 78-82% of the magnitude of the original response. Since exposing the antibodies to 62.8°C for an hour should deactivate them, a response to DMMP was not expected. Hence, a response of 78-82% of the original response could possibly be due to the protein coating and not the antibody activity.

VI. HUMIDITY EFFECT

a. Humidity Studies

The performance of the protein coated detector was evaluated at humidity levels up to 42% (Figure 15). Response decreased as the humidity level increased. Although the response in the dry phase was about 15% higher than that at the lowest humidity level studied. A useful calibration curve at 5% relative humidity

level could be obtained (Figure 16). Also, the magnitude of the response at 40% relative humidity for the highest concentration delivered by the permeation device was only 22% of that obtained at 5% relative humidity.

The response at greater than 40% relative humidity was minimal because the coating became saturated with water and analyte. To minimize the moisture effect, some hydrophobic materials (PVC, Silicon) were mixed with the coating or were cast as a thin film over the coating to repel water molecules. This did not produce any significant improvement and the response was completely lost in the case of casting a protective film due to the obvious lack of diffusion of the organophosphorus compound through the film.

b. Scrubber Evaluation

Several scrubbers were then evaluated. The adsorption of DMMP by various desiccants varied from 100% in the case of $CaSO_4$ to only 10% with Na_2SO_4 The evaluation of the most promising desiccants is shown in Table 22. It was concluded that Na_2SO_4 did not seriously affect the sample integrity and response and can be used in practical applications to remove moisture prior to assay.

VII. SELECTIVITY

Interferences from various substances expected as potential interferants were tested. Water, gasoline and sulfur fumes were tested by a continuous process method. Other trace gases,

including SO_2, NH_3, HCl, $COCl_2$, CO, H_2S and auto exhaust, were tested by the syringe dilution method.

Water, auto exhaust, sulfur and gasoline fumes caused high frequency changes in comparison to those obtained by the highest concentration of DMMP tested (2.00 ppm). The effects of these interferants were eliminated by passing the sampling stream through a column of sodium sulfate as mentioned earlier in the humidity study to be effective in reducing large moisture levels.

The selectivity of BSA, IgG and anti-DMMP coatings toward DMMP was then evaluated. All three coatings responded favorably to DMMP vapor. BSA yielded the highest response of all three by 10-15%. Fifty percent of the time a coating of BSA and glutaraldehyde, in the ratio, of greater than 10%, used as a reference coating material, gave a higher response to DMMP than the antibody or enzyme coatings. As with the antibody coatings, the more mass added to the crystal, the higher the response. It appeared after reviewing all of the data that most protein coatings tested would be sensitive to DMMP.

DIMP gave a higher response than DMMP with GD-1 and anti-DMMP coatings. This was attributed to the fact that DIMP has a higher molecular weight than DMMP.

Dextran, BSA, and anti-DMMP were also evaluated for a response to four pesticides which have similar functional groups: malathion, ethion, methyl parathion and D I syston. The antibody coated crystals and the BSA coated crystals were sensitive to both ethion and methyl parathion. Ethion gave a response which

was approximately 60% of the response to DMMP. Methyl parathion yielded a response 30% of a DMMP response. The antibody coatings were also inhibited 70% by D I syston. BSA coatings yielded a response of 60% and 37% to ethion and methyl parathion, respectively. Malathion had less than a 20% response for any of the coatings.

It was concluded that the antibody coatings could only be considered class specific for organophosphorus compounds not specific for DMMP only.

VIII. REPRODUCIBILITY, STABILITY AND LIFETIME

a. Enzyme & BSA Coating

The response of all enzyme coated crystals was initially high, then decreased and stabilized after the first three measurements. Although no consistent response was noticed, it was believed that some kind of interaction between the active enzyme and DMMP occurs during the initial exposures. The enzyme might have lost some of its activity and later responses would partially be attributed to merely the absorption of DMMP by the total protein coating. The reproducibility of both enzyme and BSA coated crystals were quite good, exhibiting responses with approximately a 5% relative standard deviation within the same day. The useful lifetime of a single coating was determined to be at least one month (750 determinations) with no significant decrease in sensitivity. Several coated crystals were divided into three groups; one group was stored in the refrigerator,

another group was stored in a desiccator and the third group was deliberately left on the bench exposed to the atmosphere. The response of these crystals was evaluated periodically. The performance of the third group was as favorable as those stored in the desiccator or in the refrigerator, even after three months. The shelf-lifetime of a coated crystal, stored dry, should exceed six months.

b. GD-1 Antibody & BSA Coating

Antibody coated crystals and their respective reference crystals were evaluated for reproducibility over a continuous two hour period, measuring responses to DMMP every ten minutes. All of the crystals exhibited good reproducibility (RSD=5.2% for 100% sample and 1.73% for 17% sample using five measurements) regardless of sample generation method. Although the responses using the bubbler method were higher than those using the diffusion method, small variances from one measurement to the next were obtained using the diffusion generation method.

Larger variances were noted between daily measurements where a host of variables came into play. The antibody coatings were sensitive to temperature and humidity variation. Also, a dramatic decrease in response could have been due to the loss of some of the coating from the flow of the gasses over the crystals. The crystals were usually rendered inactive after one month.

The performance of the GD-1 antibody coated crystals that showed any response to DMMP was evaluated with respect to

stability. On the average, these crystals lasted at least one to two weeks. A few remained active for almost a month, but the majority lasted at the most for two to three weeks.

Figures 17, 18 and 19 show short term reproducibility studies for a few of the crystals. These curves were obtained by making at least 8 repeated exposures to 0.9 ppm DMMP over a two to three hour period. Figures 20 and 21 show the longer term reproducibility of other crystal pairs coated at different times, but monitored over a number of days and weeks. In Figure 20, studies were conducted over a thirty day period. There was a significant decrease in the magnitude of the frequency difference between the active and the inactive crystals, but this decrease affects both crystals.

Figure 21 shows another crystal pair with about the same magnitude of response over a twenty-five day period. Both the active and the inactive crystal responses were very reproducible.

c. Anti-DMMP Antibody & BSA Coatings

The anti-DMMP coated crystals show reasonably good stability over 25-36 days. Usually the first two days exhibit the highest responses. The response usually levels off with subsequent measurements. This phenomenon was assumed to be the deactivation of the antibody, but it could also be attributed to the loss of some of the coating from the constant flow of air on the crystals. Sharp increases in response could have been due to a temperature fluctuation or storage of the crystal for more than one week in the desiccator before measuring.

Figures 22 and 23 show stability curves obtained with the anti-DMMP antibody. Figure 24 shows two sets of crystals with fairly good reproducible responses to DMMP.

IX. FINAL PROTOTYPE

The PZ-105 gas phase detector final prototype has been completed. It is microprocessor controlled and engineered to work with a wide range of coated piezoelectric crystals. It features: dual crystal chamber (reference & test), frequency counter, air pump, temperature sensor, relative humidity sensor, digital display, analog concentration output, RS-232 output, alarm buzzer, and battery operation. The specifications are as follows:

Oscillator	5 to 15 MHz crystals
Frequency resolution	1 Hz
Frequency accuracy	+1%
Frequency difference range	-32,768 to +32,767 Hz
Air flow maximum	> 100 mL/min
Temperature range	-20 to +50°C
Temperature resolution	1°C
Temperature accuracy	+3°C
Relative humidity range	10 to 99% RH
Relative humidity resolution	1% RH
Relative humidity accuracy	+3% RH
Analog output	0 to 1 V, in 5 concentration ranges
Concentration range	0 to 32,767
Digital output	RS-232
Power supply	115 V, 50-60 Hz
Weight	5.5 lbs (2.5 kg)

Physical dimensions	9.5 x 26 x 28 cm (h,w,d) without handle
	9.5 x 30 x 28 cm (h,w,d) with handle
Crystal posts	0.050 in (1.27 mm) diameter
	0.25 in (6.35 mm) long 0.486 in (12.34 cm) between posts

6. CONCLUSION

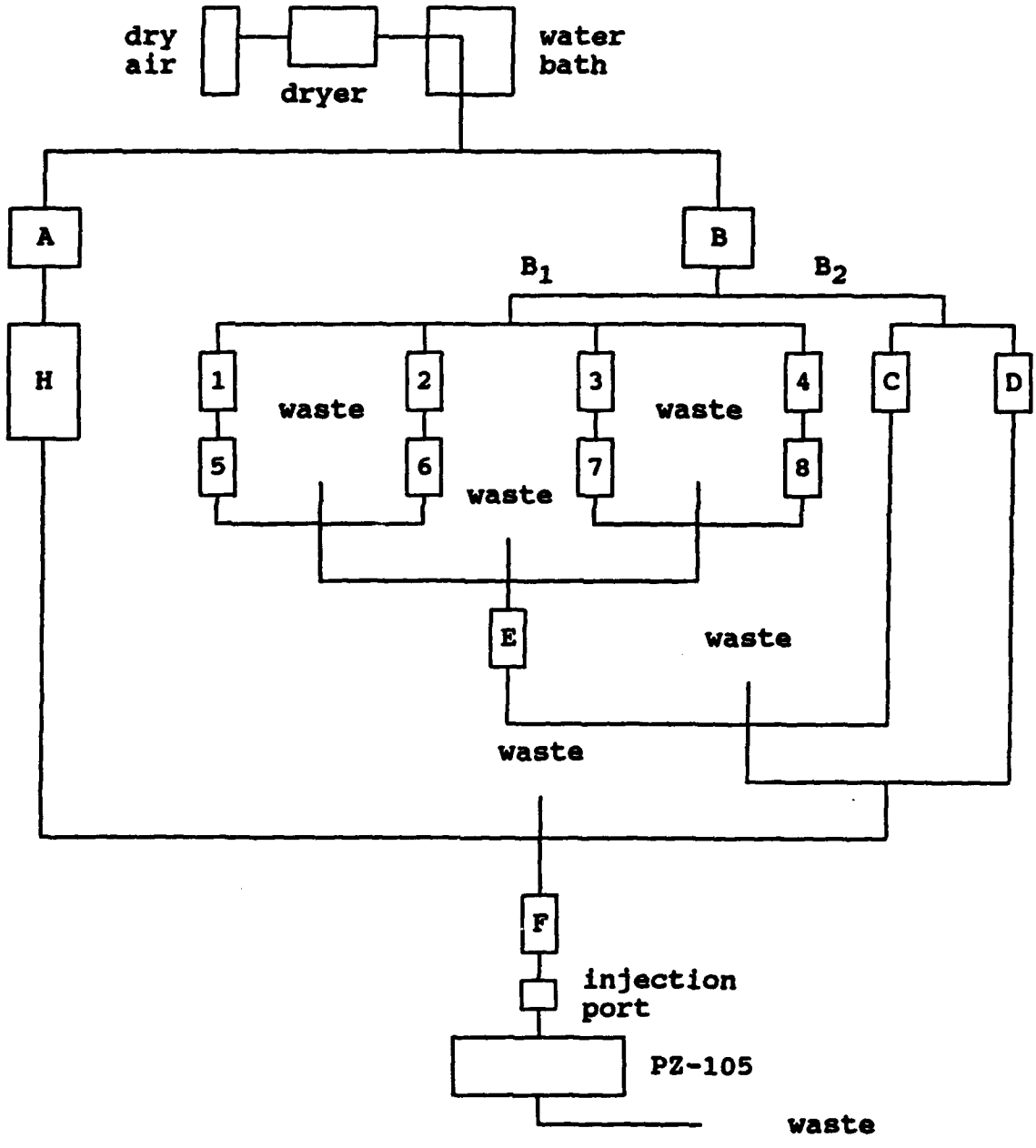
A prototype piezoelectric crystal detector was developed during this project for the detection and determination of organophosphates.

The instrument is microprocessor controlled, with optional battery operation and RS-232 output. The dimensions of the instrument are 9.5 x 30 x 28 cm. (with the handle) and weighs 5.5 pounds. It incorporates humidity and temperature sensors and an alarm to alert to dangerous levels of exposure.

The protein coatings evaluated, acetylcholinesterase, GD-1 anti-soman, anti-DMMP and bovine serum albumin all showed good promise when evaluated versus a dextran coating on the reference crystal.

The instrument developed in this project has many potential applications and can be used for monitoring a variety of pollutants and toxic vapors. Its usefulness is limited only by the inability to develop a selective coating material.

FIGURE 1



A) Humid air stream
B) Dry air stream
C) Diluent flow meter
D) Background flow meter
E) Sample air stream
F) Total air stream
H) Humidifier

1) DMMP flow meter
2) DIMP flow meter
3) Interferent #1 flow meter
4) Interferent #2 flow meter
5) DMMP source
6) DIMP source
7) Interferent #1 source
8) Interferent #2 source

FIGURE 2

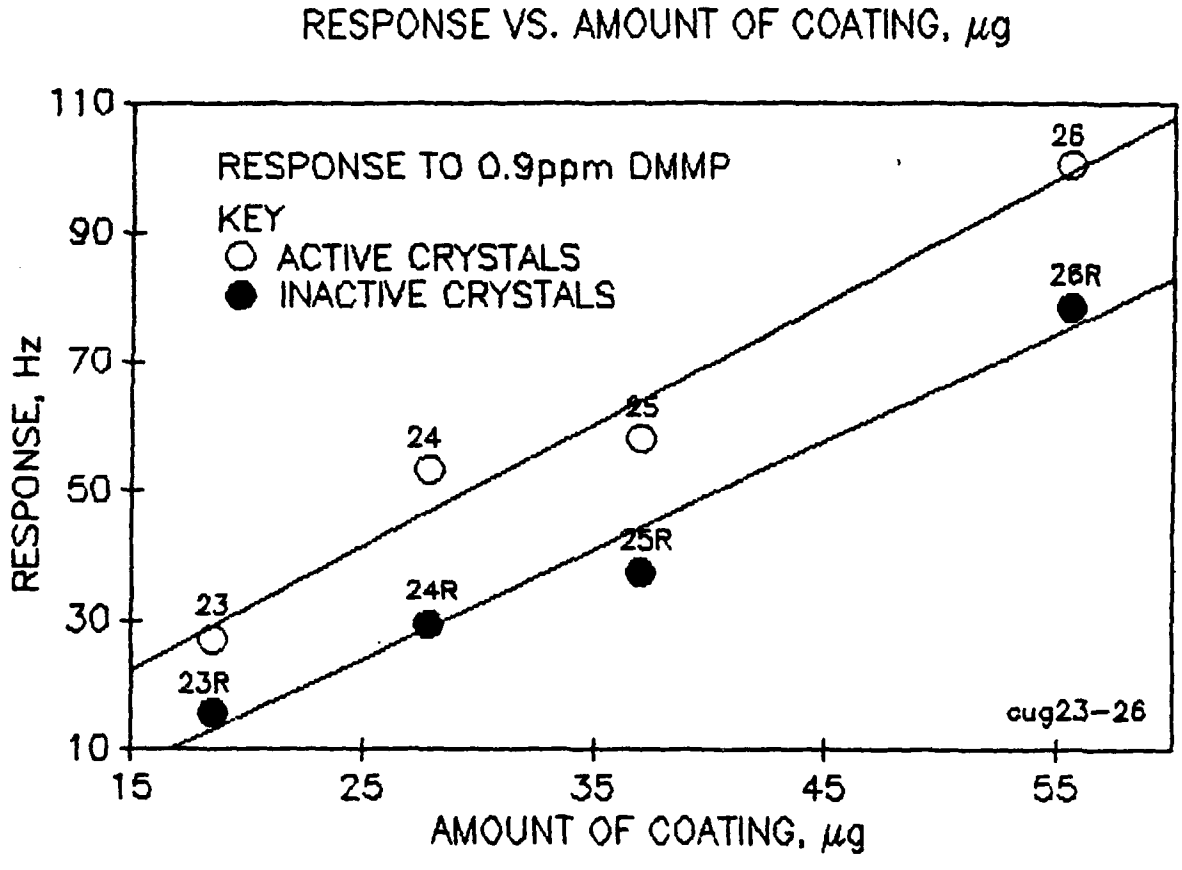

RESPONSE VS. AMOUNT OF COATING, μg
RESPONSE TO 0.9ppm DMMP
KEY
ACTIVE CRYSTALS
INACTIVE CRYSTALS
RESPONSE, Hz
110
90
70
50
30
10
15
25
35
45
55
AMOUNT OF COATING, μg
23
23R
24
24R
25
25R
26
26R
aug23-26

FIGURE 3

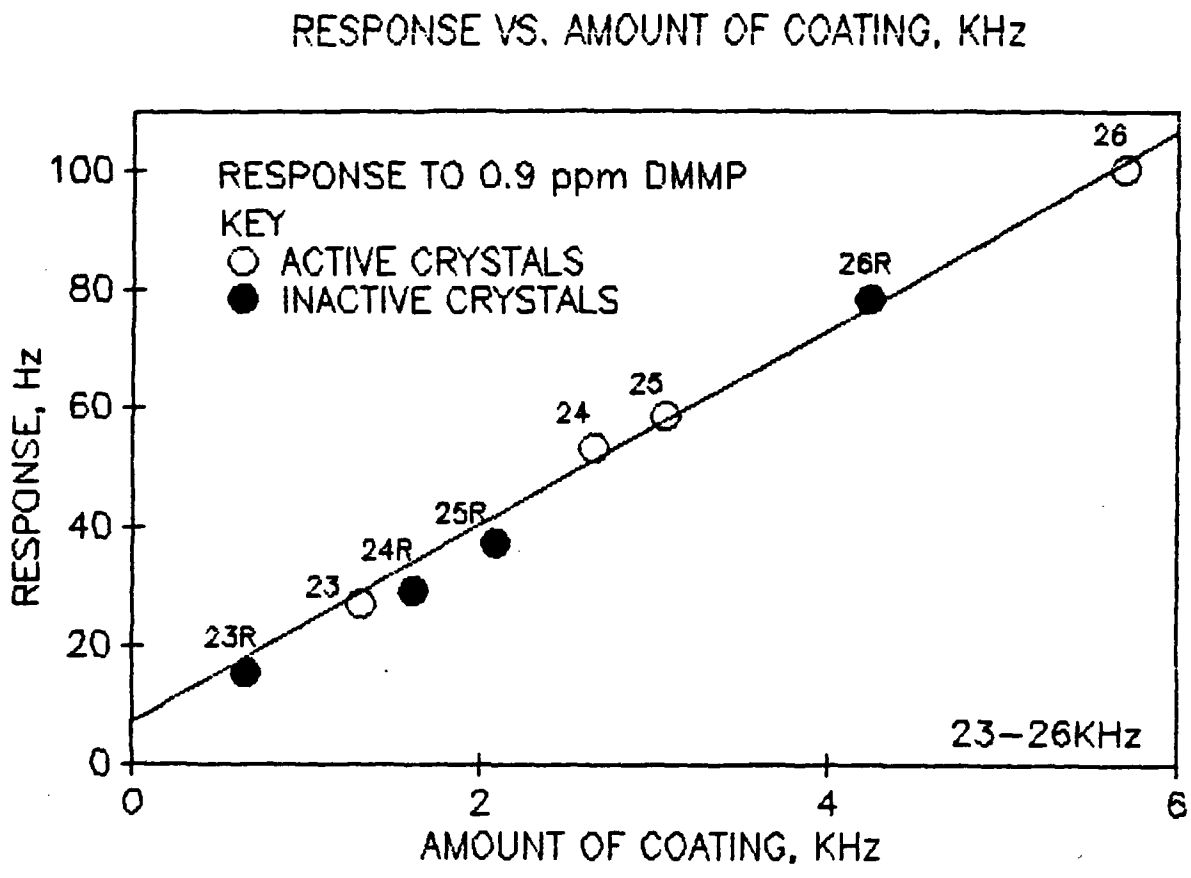

RESPONSE VS. AMOUNT OF COATING, KHz
RESPONSE TO 0.9 ppm DMMP
KEY
ACTIVE CRYSTALS
INACTIVE CRYSTALS
23R
23
24R
24
25R
25
26R
26
23-26KHz
RESPONSE, Hz
AMOUNT OF COATING, KHz
0
20
40
60
80
100
0
2
4
6

FIGURE 4

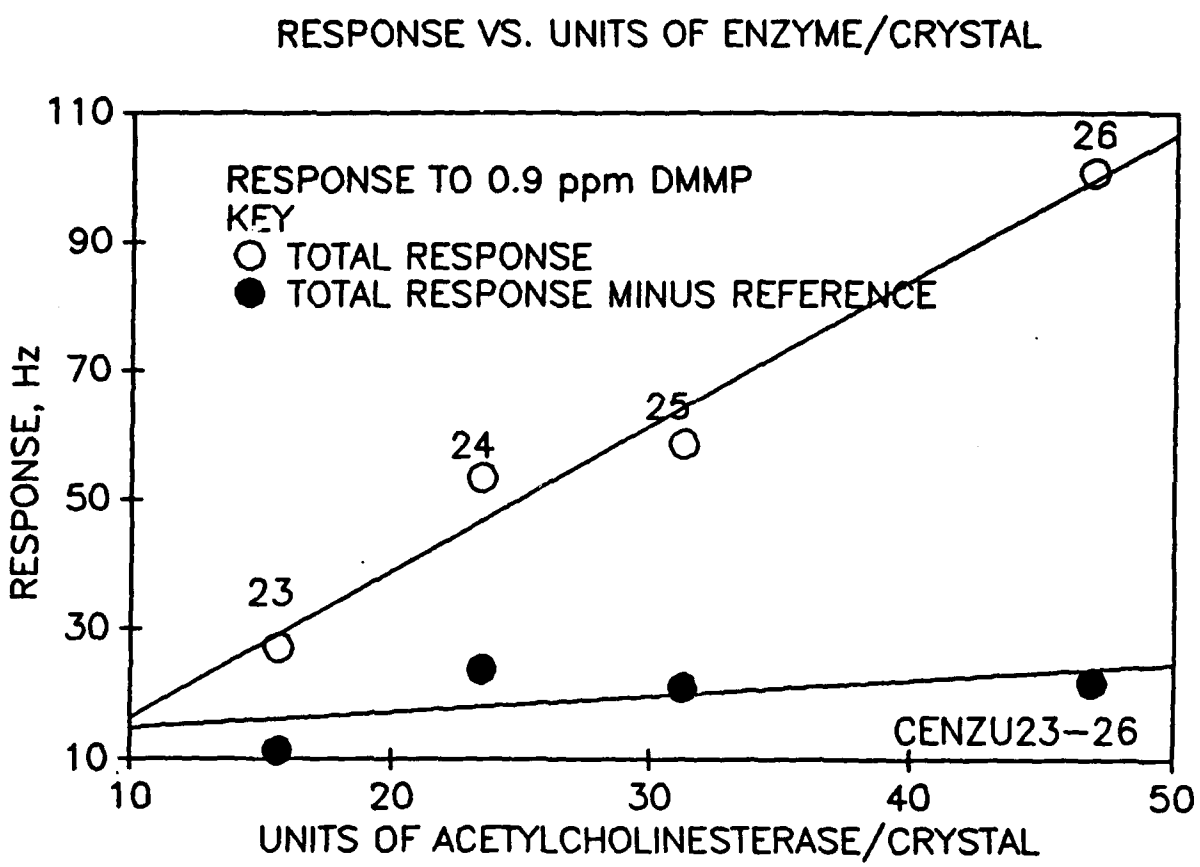

RESPONSE VS. UNITS OF ENZYME/CRYSTAL
RESPONSE TO 0.9 ppm DMMP
KEY
TOTAL RESPONSE
TOTAL RESPONSE MINUS REFERENCE
RESPONSE, Hz
110
90
70
50
30
10
23
24
25
26
CENZU23-26
10
20
30
40
50
UNITS OF ACETYLCHOLINESTERASE/CRYSTAL

FIGURE 5

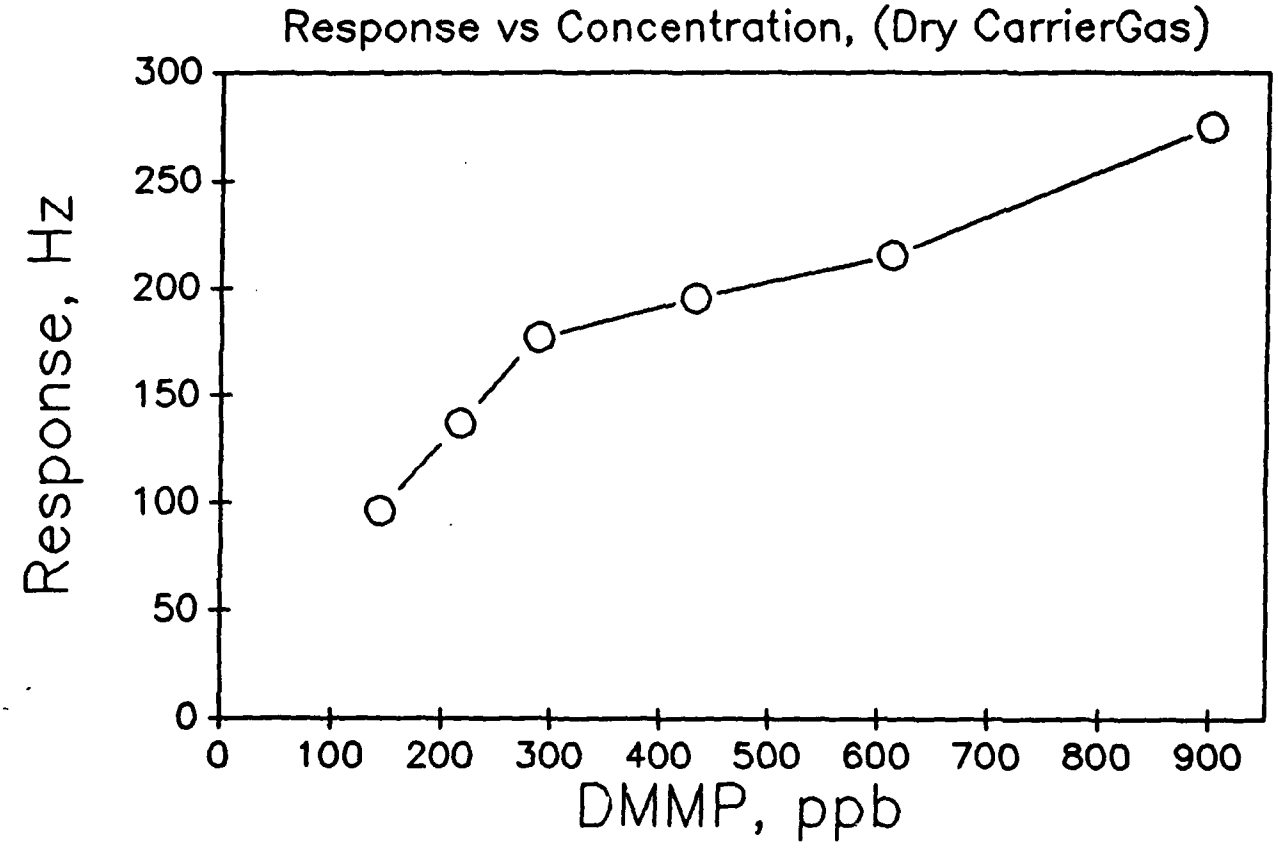

Response vs Concentration, (Dry CarrierGas)
Response, Hz
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
900
DMMP, ppb

FIGURE 6

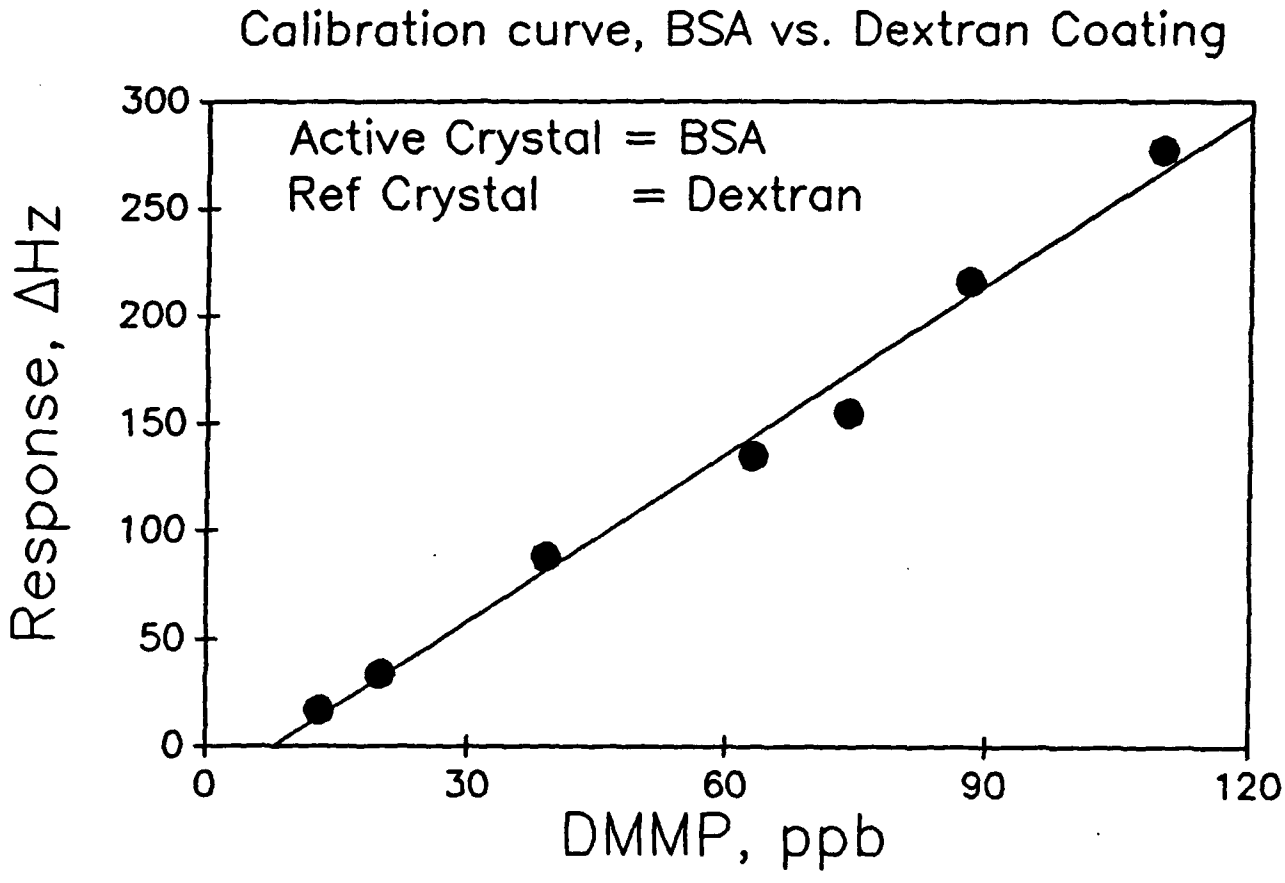

Calibration curve, BSA vs. Dextran Coating
Active Crystal = BSA
Ref Crystal = Dextran
Response, ΔHz
300
250
200
150
100
50
0
0
30
60
90
120
DMMP, ppb

FIGURE 7

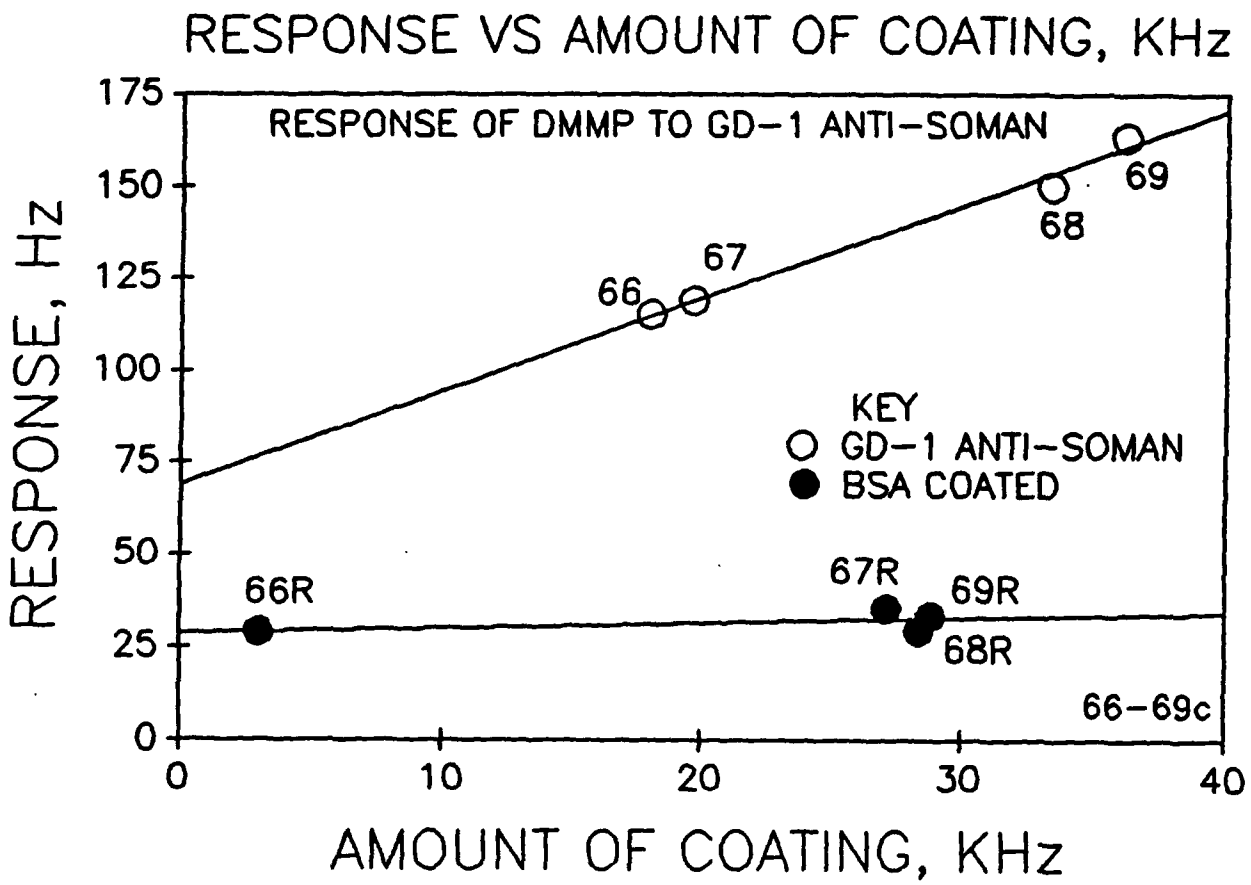

RESPONSE VS AMOUNT OF COATING, KHz
RESPONSE OF DMMP TO GD-1 ANTI-SOMAN
RESPONSE, Hz
175
150
125
100
75
50
25
0
66
67
68
69
KEY
GD-1 ANTI-SOMAN
BSA COATED
66R
67R
69R
68R
66-69c
0
10
20
30
40
AMOUNT OF COATING, KHz

FIGURE 8

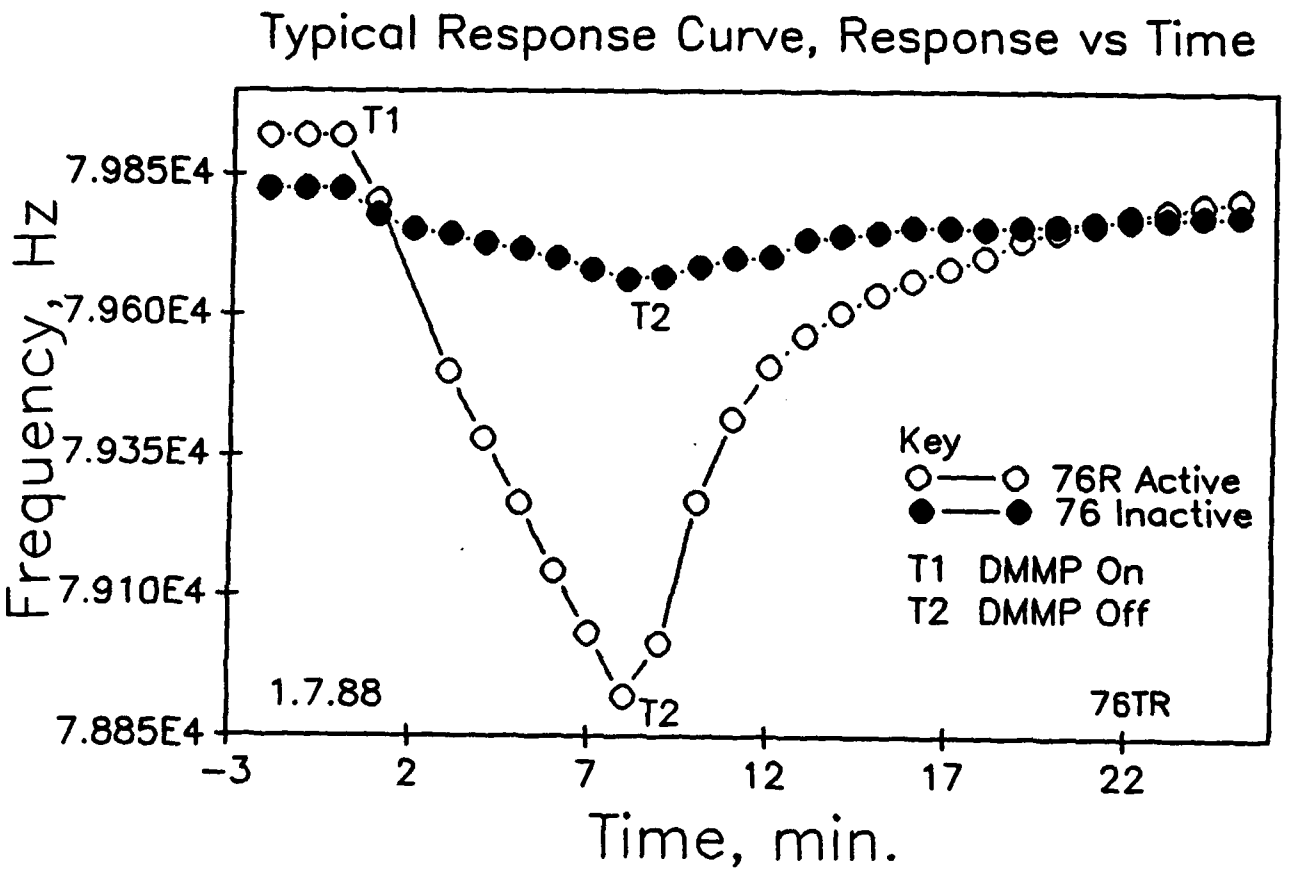

Typical Response Curve, Response vs Time
T1
T2
T2
7.985E4
7.960E4
7.935E4
7.910E4
7.885E4
Frequency, Hz
Key
76R Active
76 Inactive
T1 DMMP On
T2 DMMP Off
1.7.88
76TR
-3
2
7
12
17
22
Time, min.

FIGURE 9

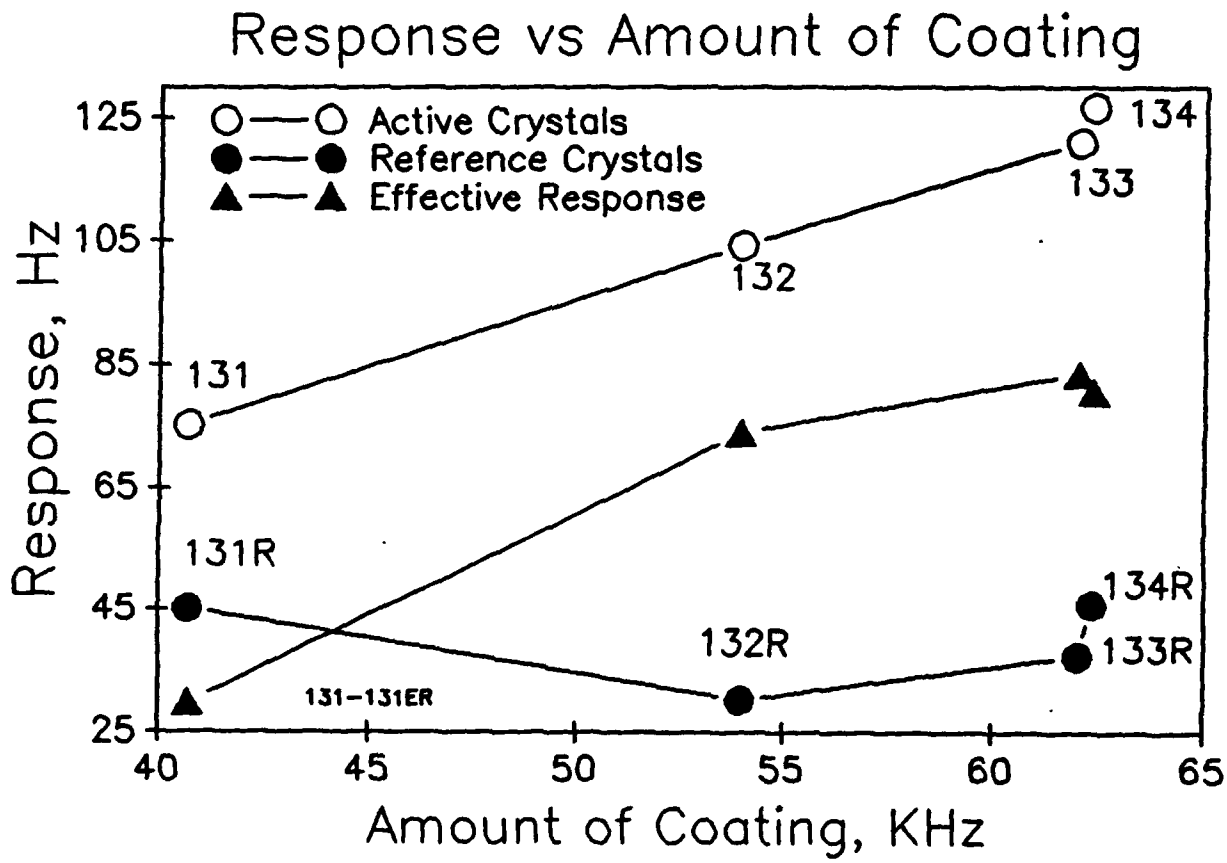

Response vs Amount of Coating
Active Crystals
Reference Crystals
Effective Response
125
105
85
65
45
25
Response, Hz
131
132
133
134
131R
132R
133R
134R
131-131ER
40
45
50
55
60
65
Amount of Coating, KHz

FIGURE 10

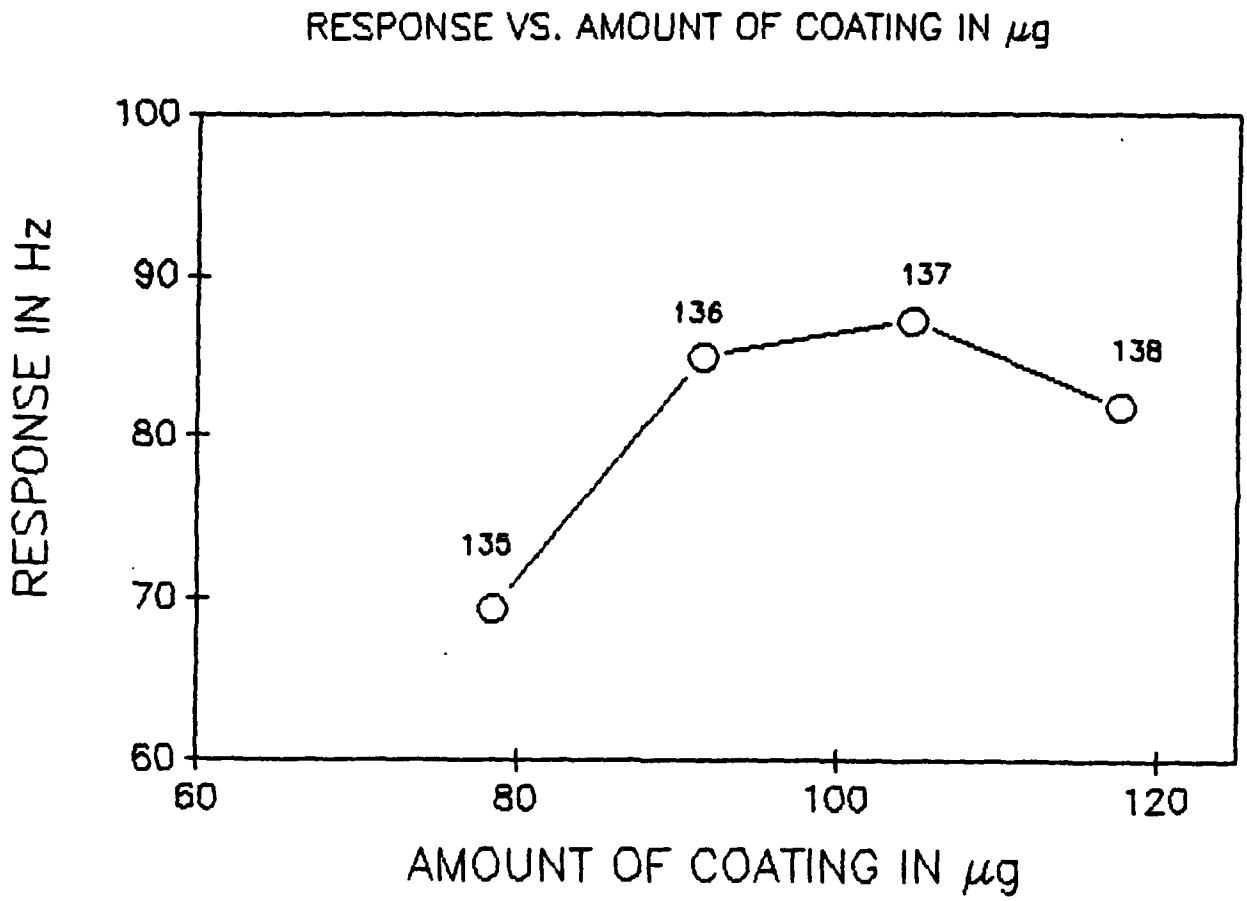

RESPONSE VS. AMOUNT OF COATING IN μg
RESPONSE IN Hz
100
90
80
70
60
135
136
137
138
60
80
100
120
AMOUNT OF COATING IN μg

FIGURE 11

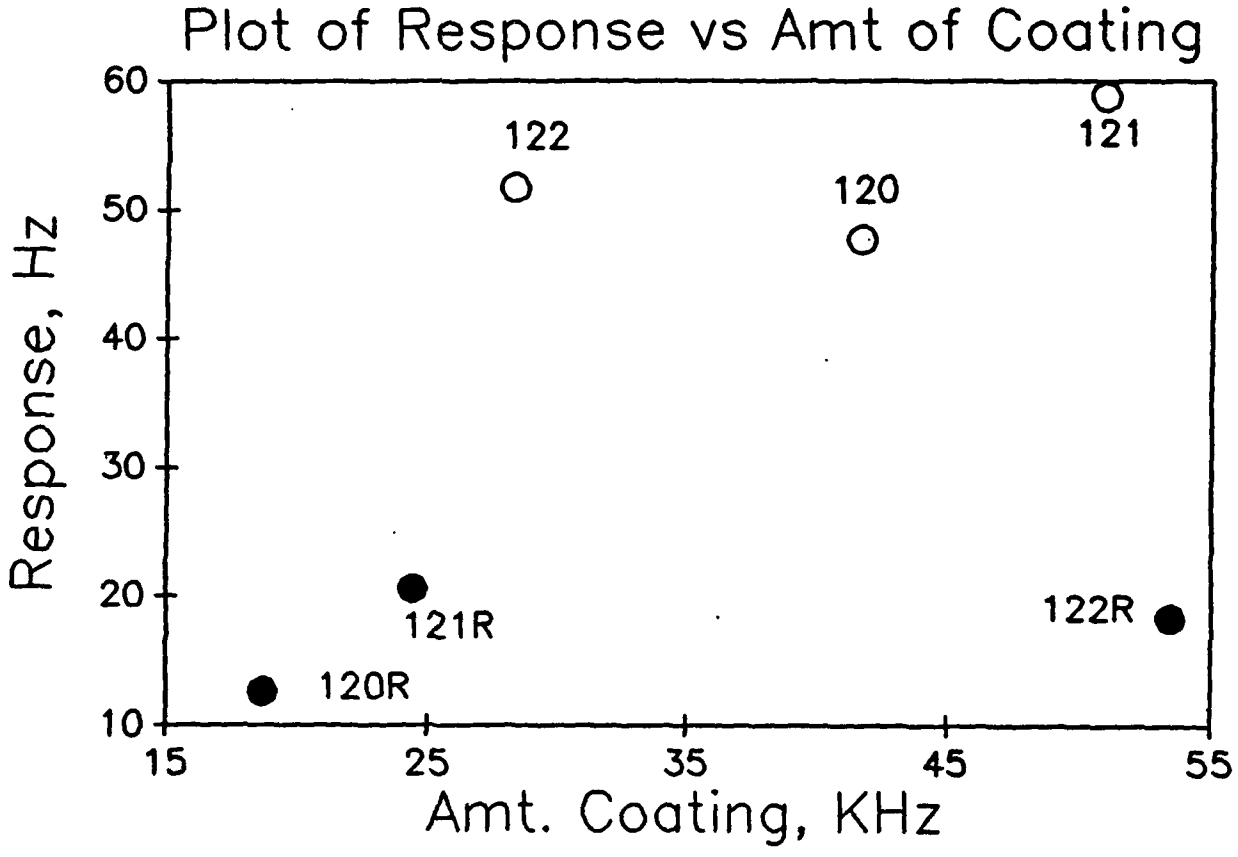

Plot of Response vs Amt of Coating
Response, Hz
60
50
40
30
20
10
15
25
35
45
55
Amt. Coating, KHz
122
121
120
121R
122R
120R

FIGURE 12

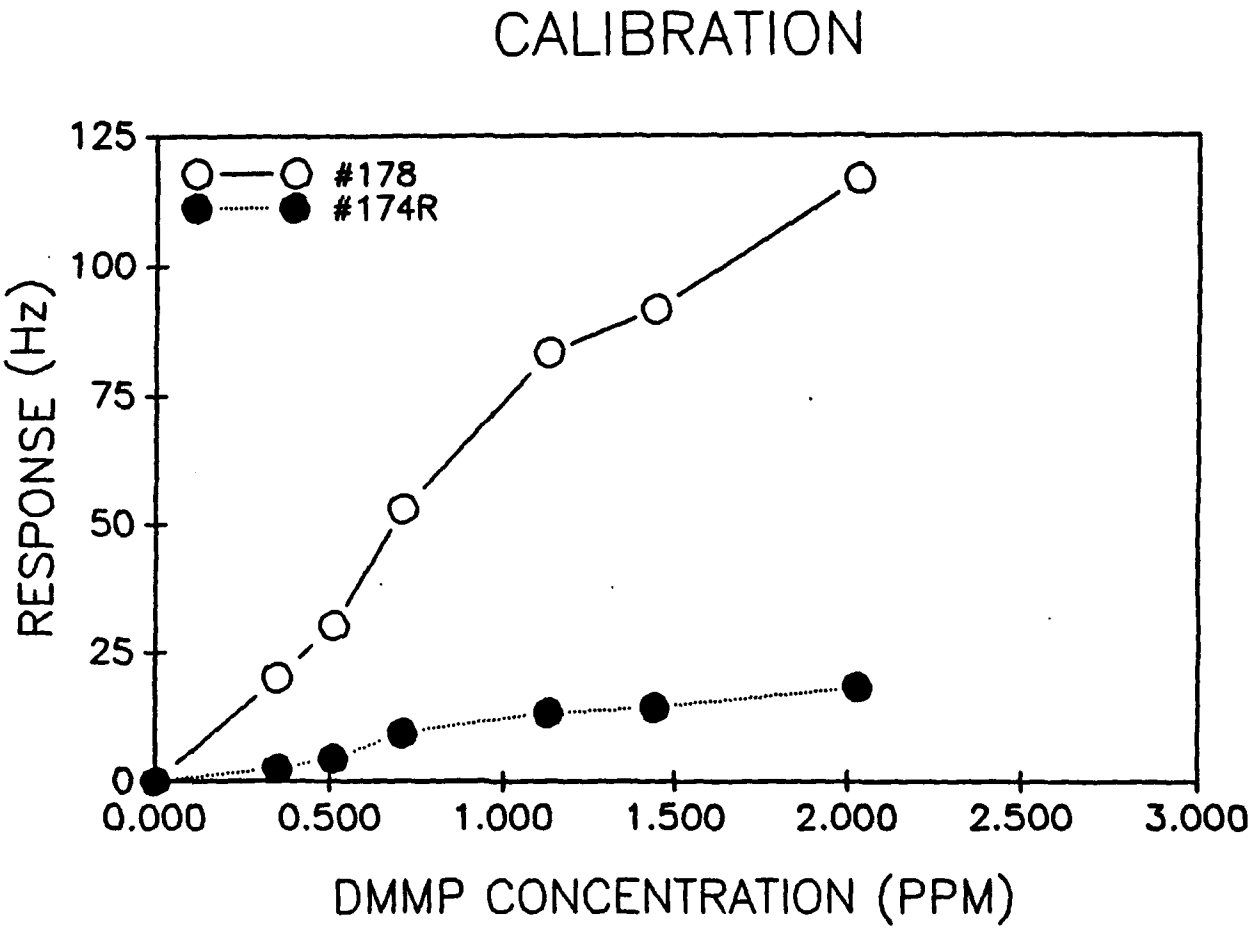

CALIBRATION
125
100
75
50
25
0
#178
#174R
RESPONSE (Hz)
0.000
0.500
1.000
1.500
2.000
2.500
3.000
DMMP CONCENTRATION (PPM)

FIGURE 13

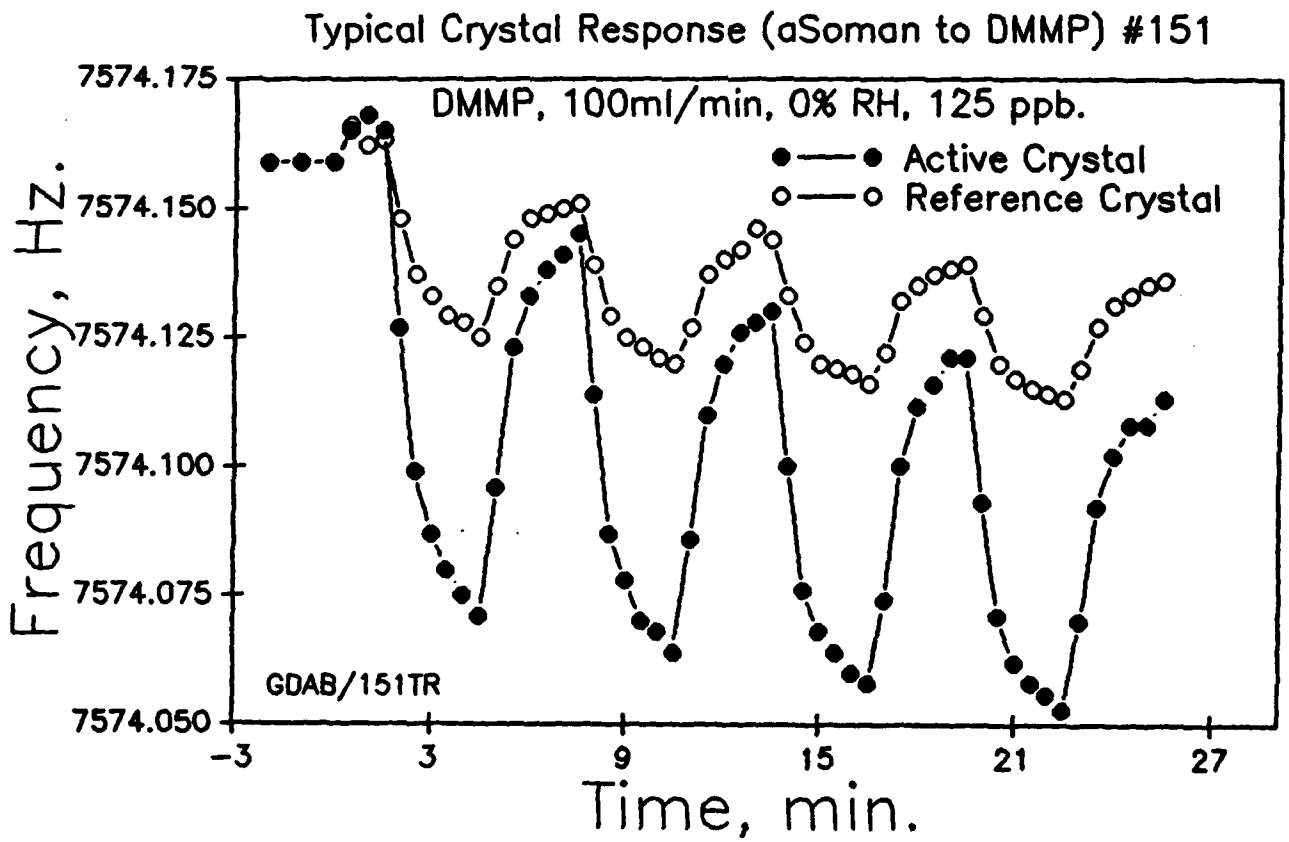

Typical Crystal Response (aSoman to DMMP) #151
DMMP, 100ml/min, 0% RH, 125 ppb.
Active Crystal
Reference Crystal
GDAB/151TR
Frequency, Hz.
7574.175
7574.150
7574.125
7574.100
7574.075
7574.050
-3
3
9
15
21
27
Time, min.

FIGURE 14

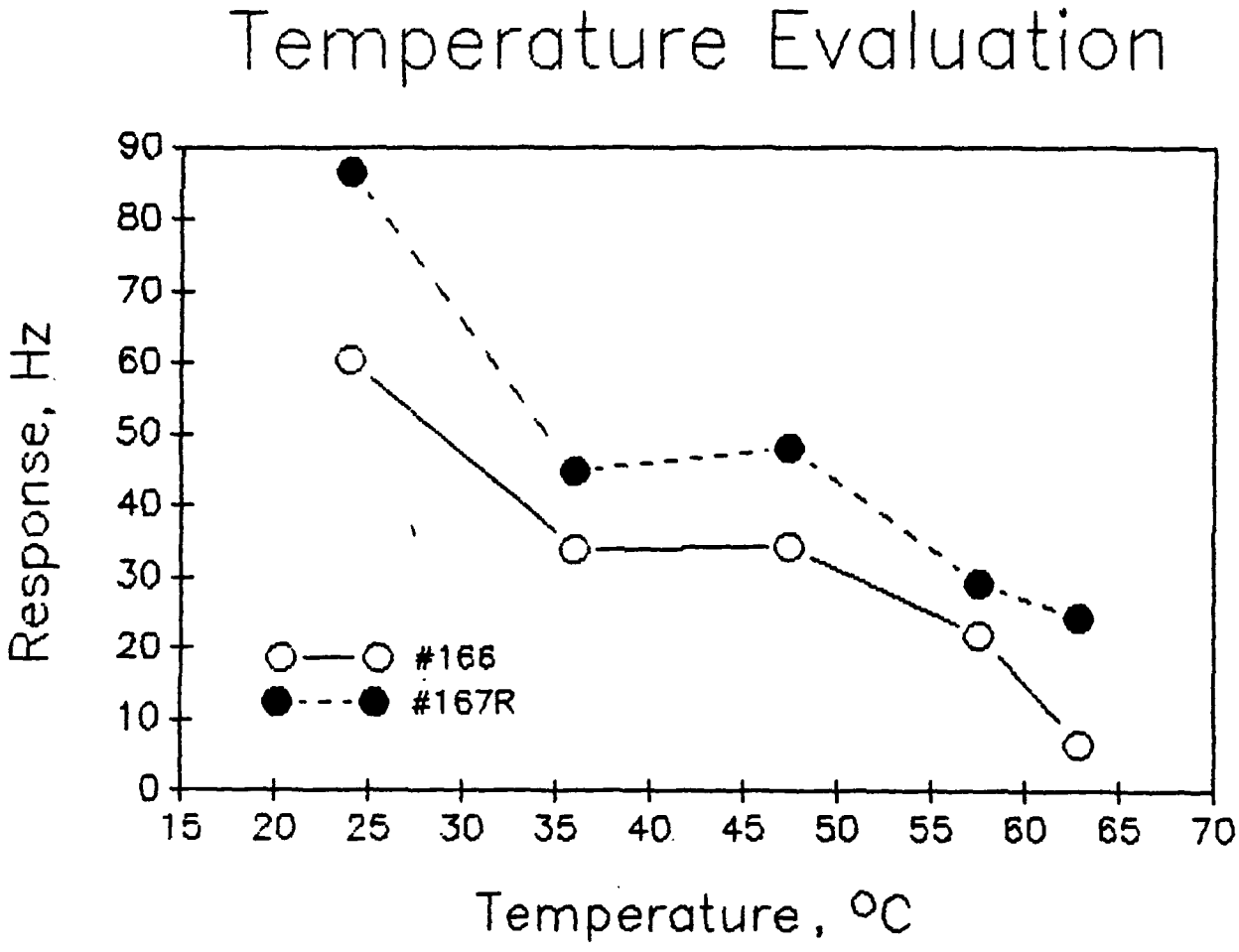

Temperature Evaluation
Response, Hz
90
80
70
60
50
40
30
20
10
0
15
20
25
30
35
40
45
50
55
60
65
70
Temperature, °C
#166
#167R

FIGURE 15

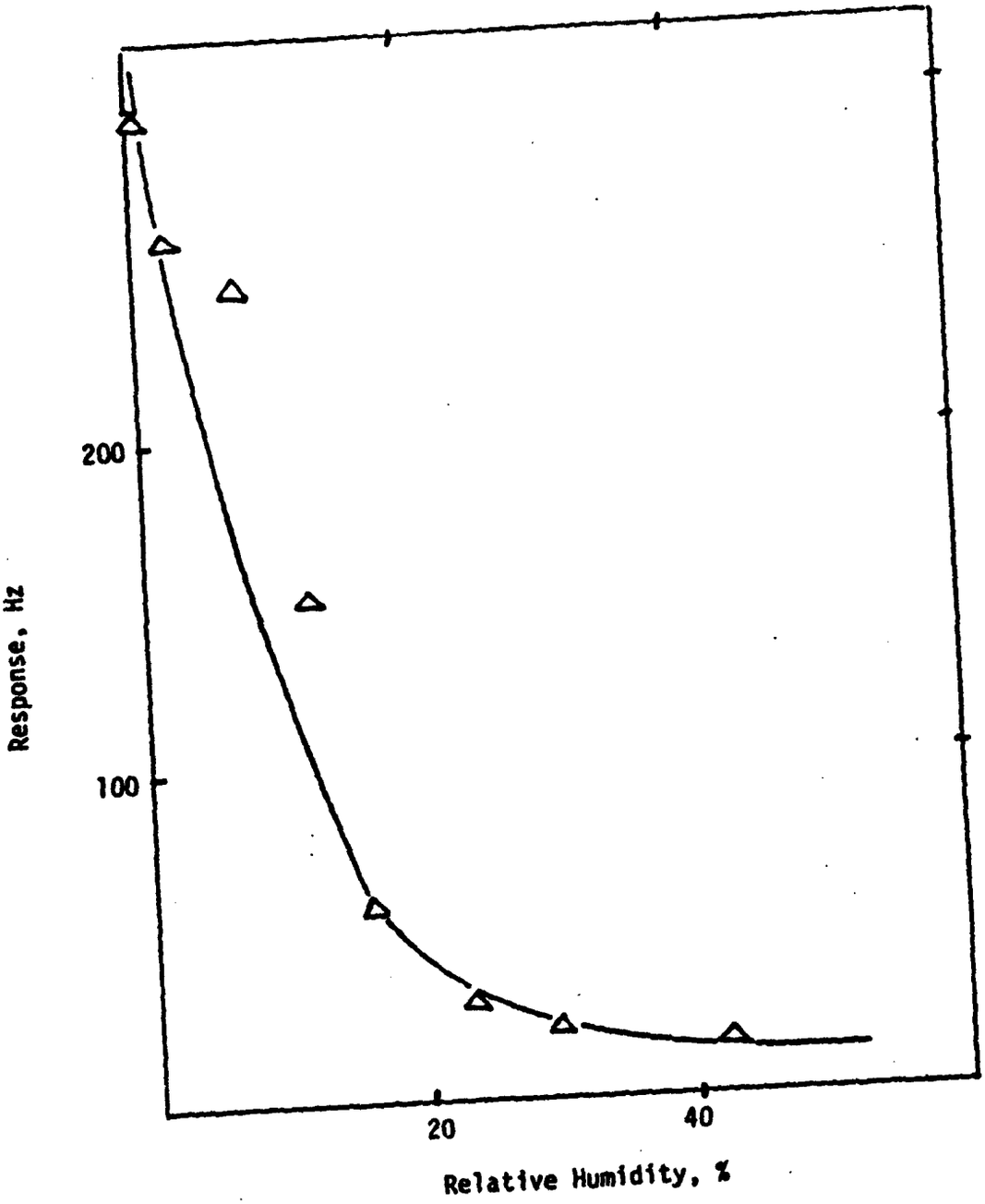


FIGURE 16

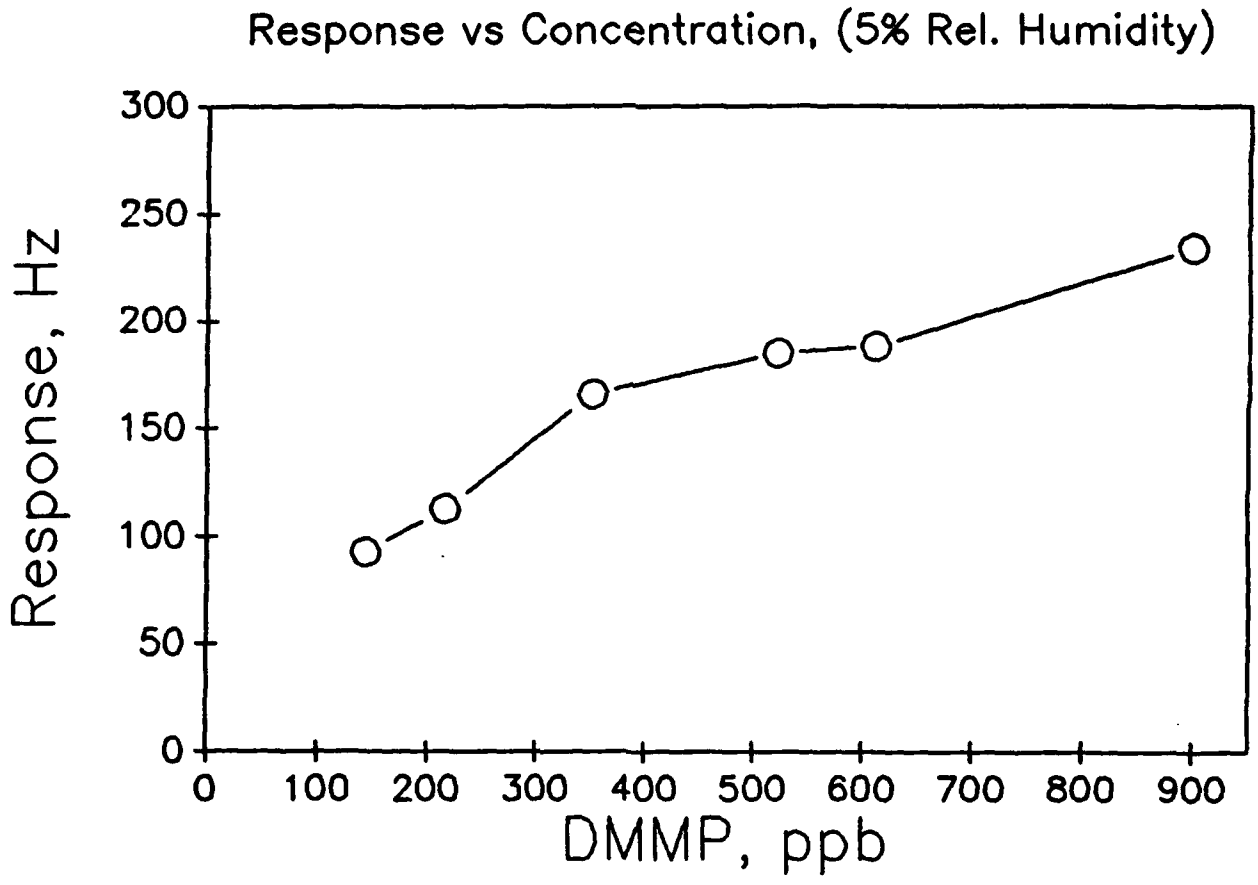

Response vs Concentration, (5% Rel. Humidity)
Response, Hz
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
900
DMMP, ppb

FIGURE 17

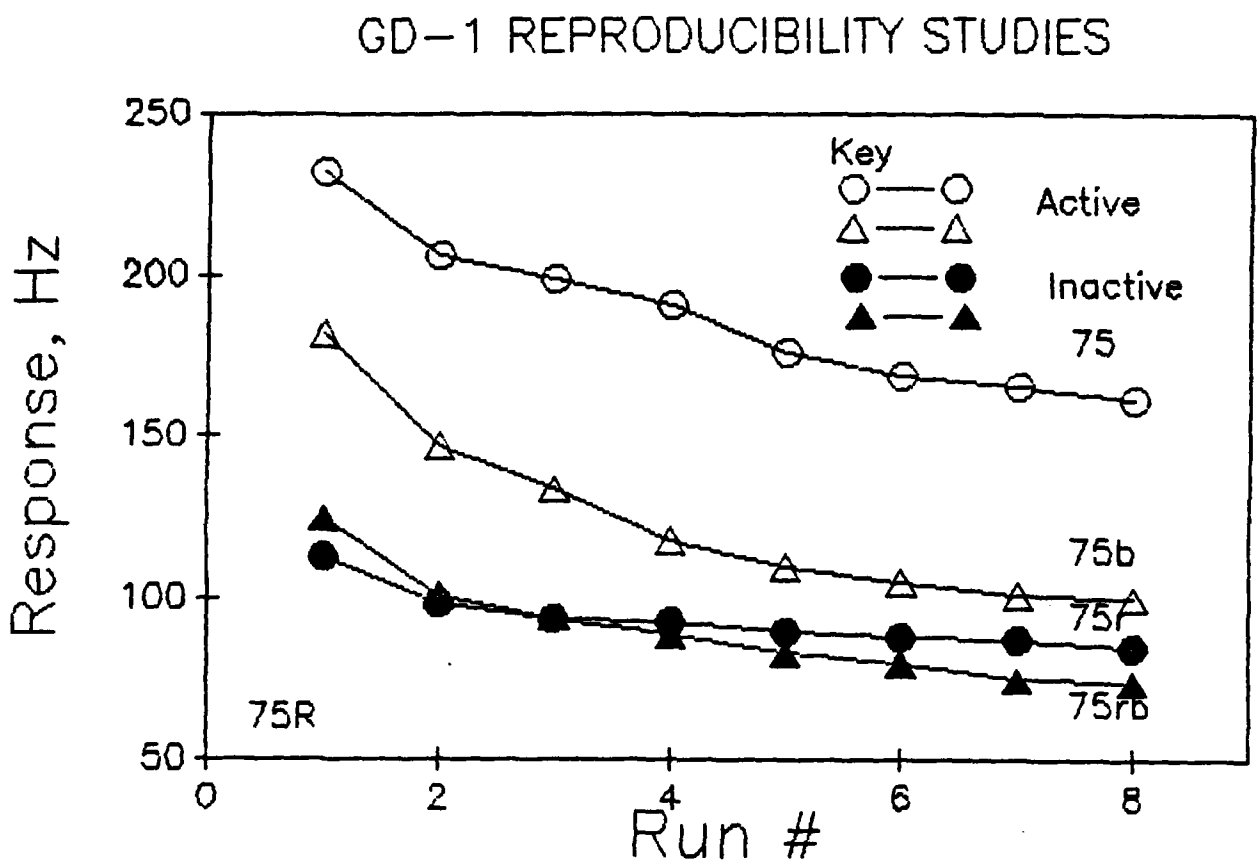

GD-1 REPRODUCIBILITY STUDIES
Response, Hz
250
200
150
100
50
0
2
4
6
8
Run #
Key
Active
Inactive
75
75b
75r
75rb
75R

FIGURE 18

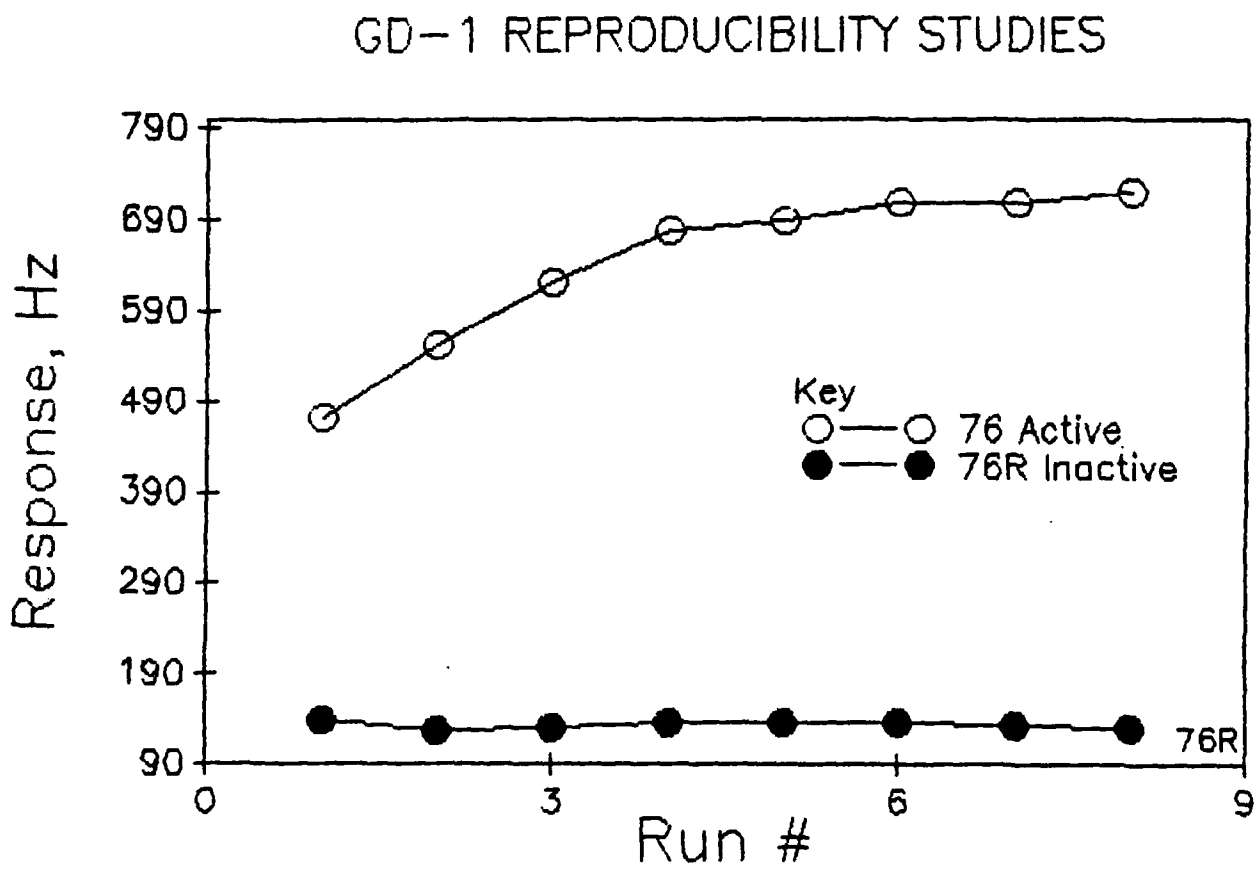

GD-1 REPRODUCIBILITY STUDIES
Response, Hz
790
690
590
490
390
290
190
90
0
3
6
9
Run #
Key
76 Active
76R Inactive
76R

FIGURE 19

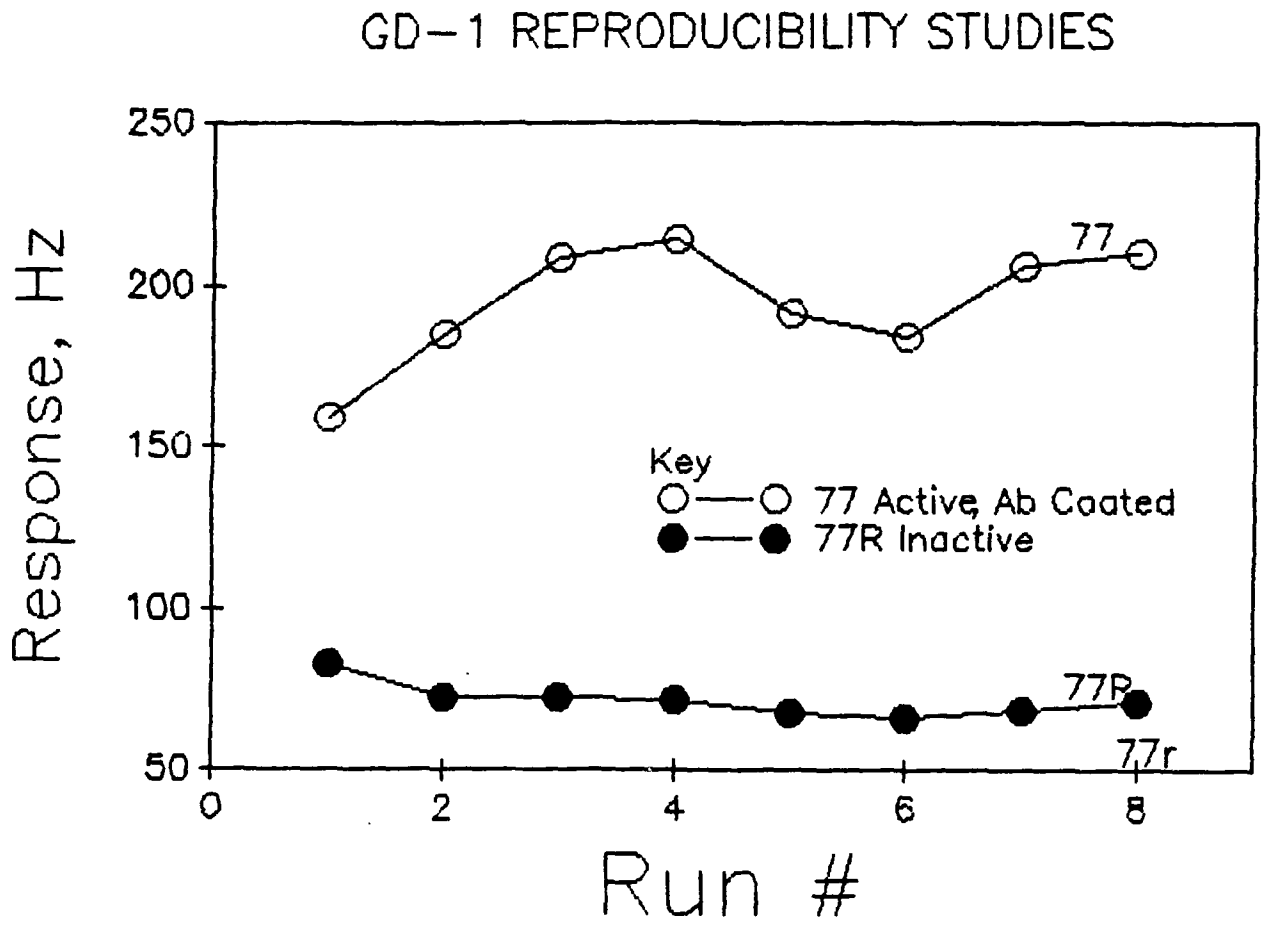

GD-1 REPRODUCIBILITY STUDIES
Response, Hz
250
200
150
100
50
0
2
4
6
8
Run #
Key
77 Active, Ab Coated
77R Inactive
77
77R
77r

FIGURE 20

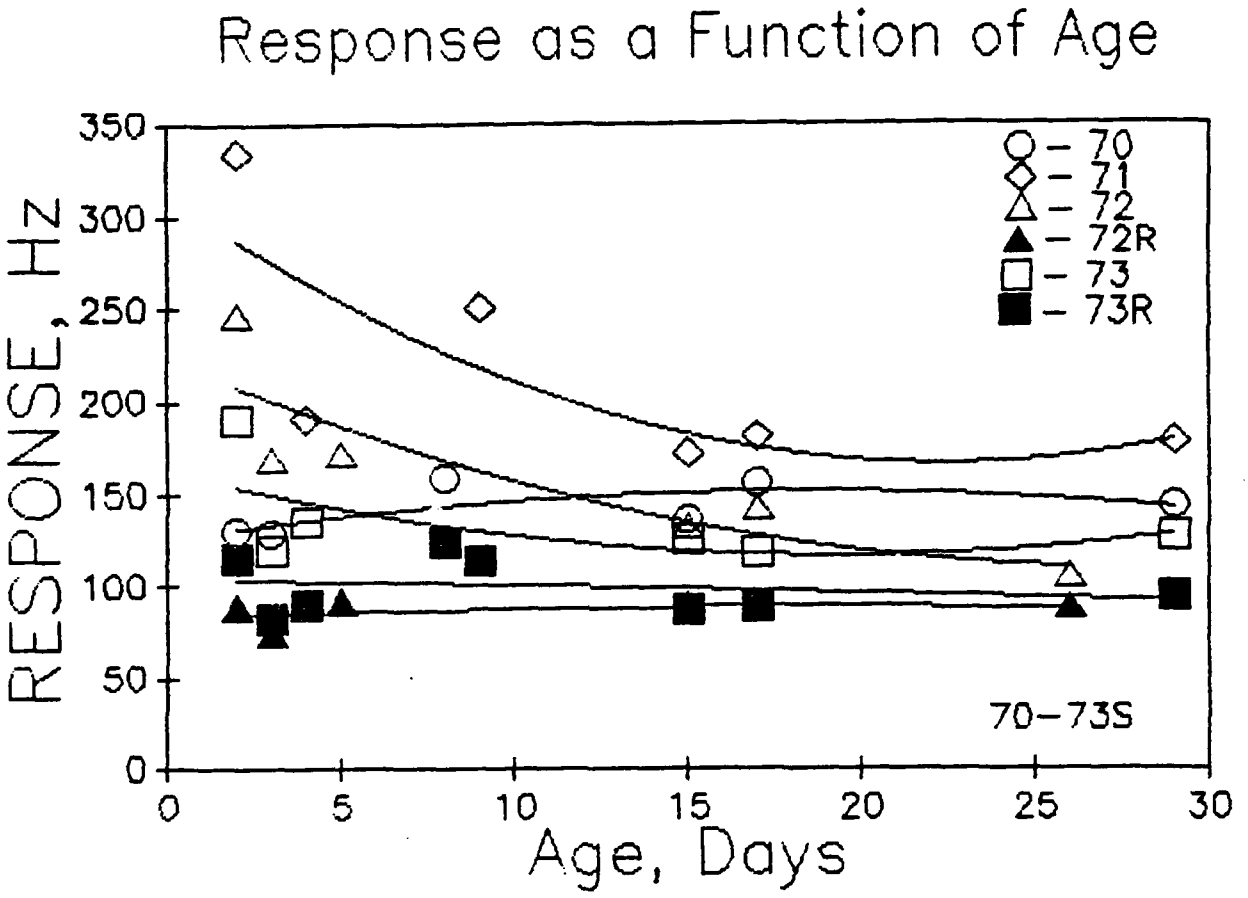

Response as a Function of Age
RESPONSE, HZ
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Age, Days
70
71
72
72R
73
73R
70-73S

FIGURE 21

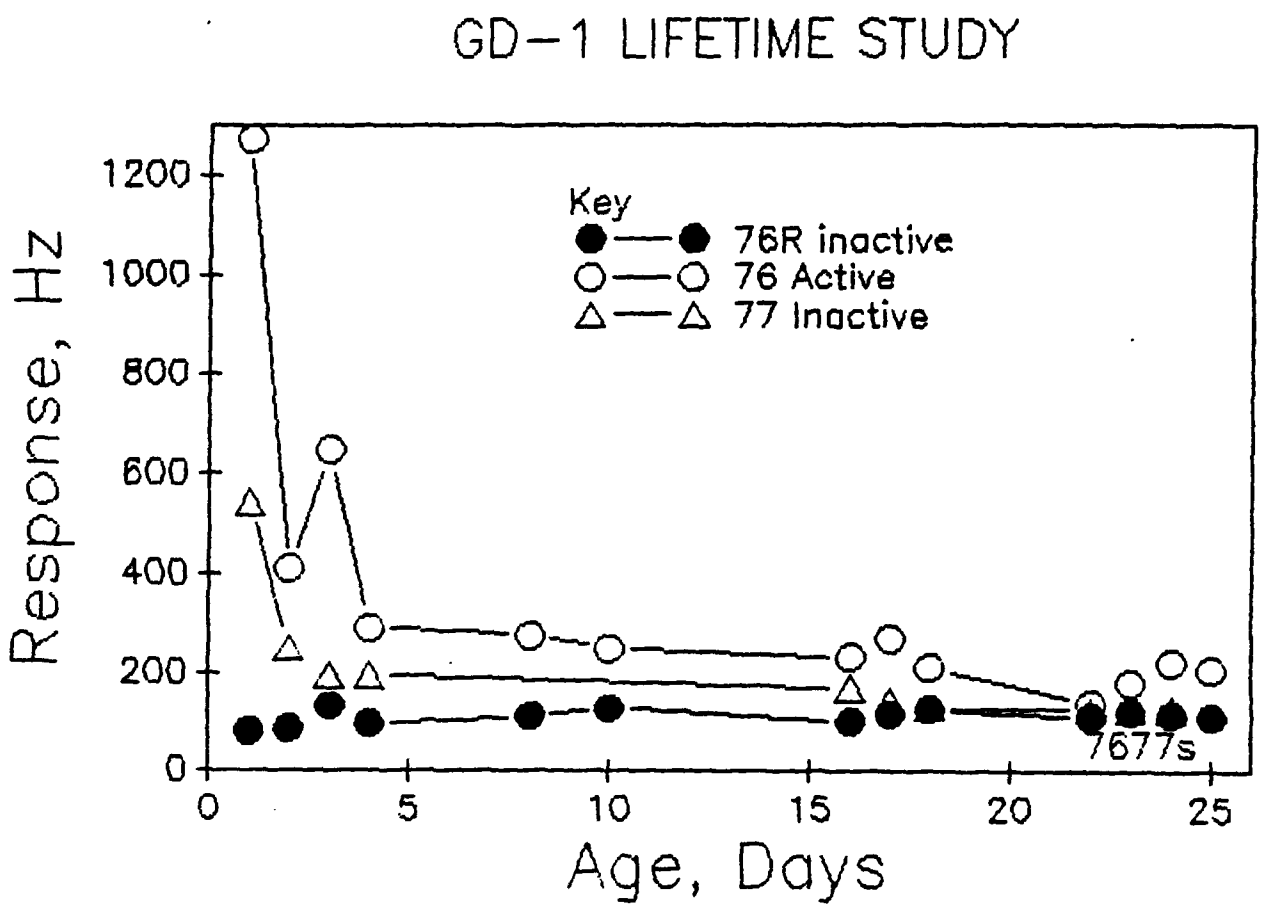

GD−1 LIFETIME STUDY
Response, Hz
1200
1000
800
600
400
200
0
Key
76R inactive
76 Active
77 Inactive
7677s
0
5
10
15
20
25
Age, Days

FIGURE 22

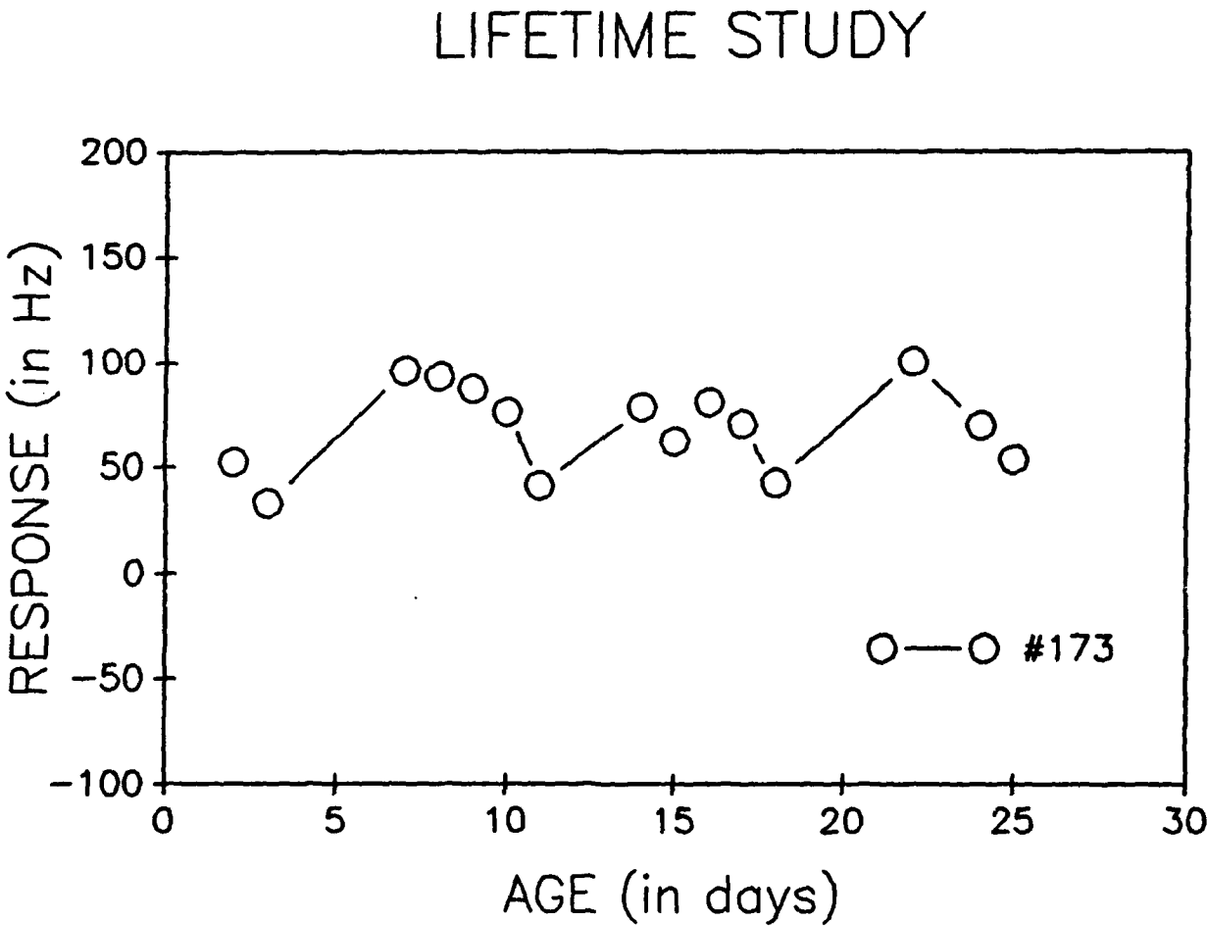

LIFETIME STUDY
RESPONSE (in Hz)
200
150
100
50
0
−50
−100
0
5
10
15
20
25
30
AGE (in days)
#173

FIGURE 23

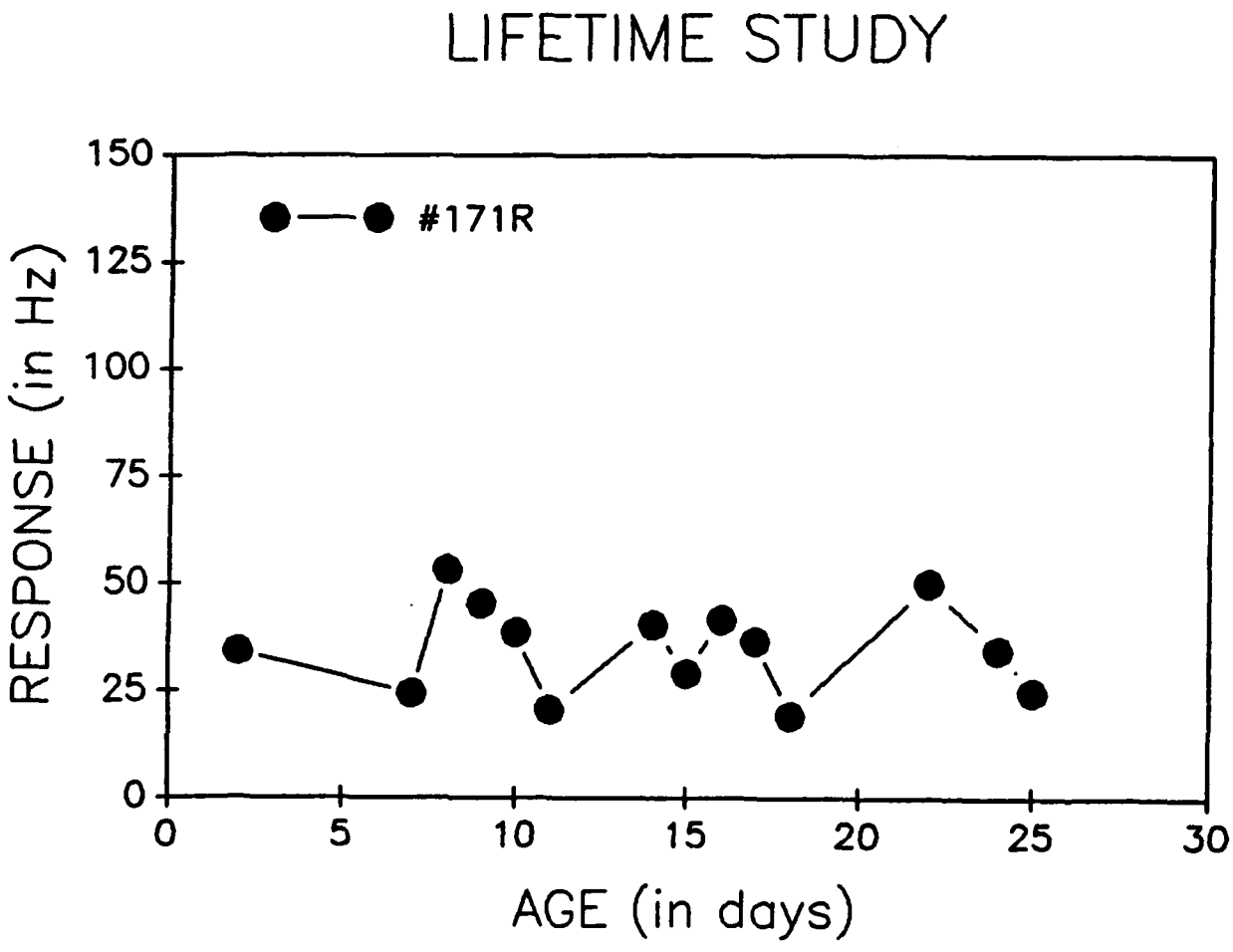

LIFETIME STUDY
#171R
RESPONSE (in Hz)
150
125
100
75
50
25
0
0
5
10
15
20
25
30
AGE (in days)

FIGURE 24

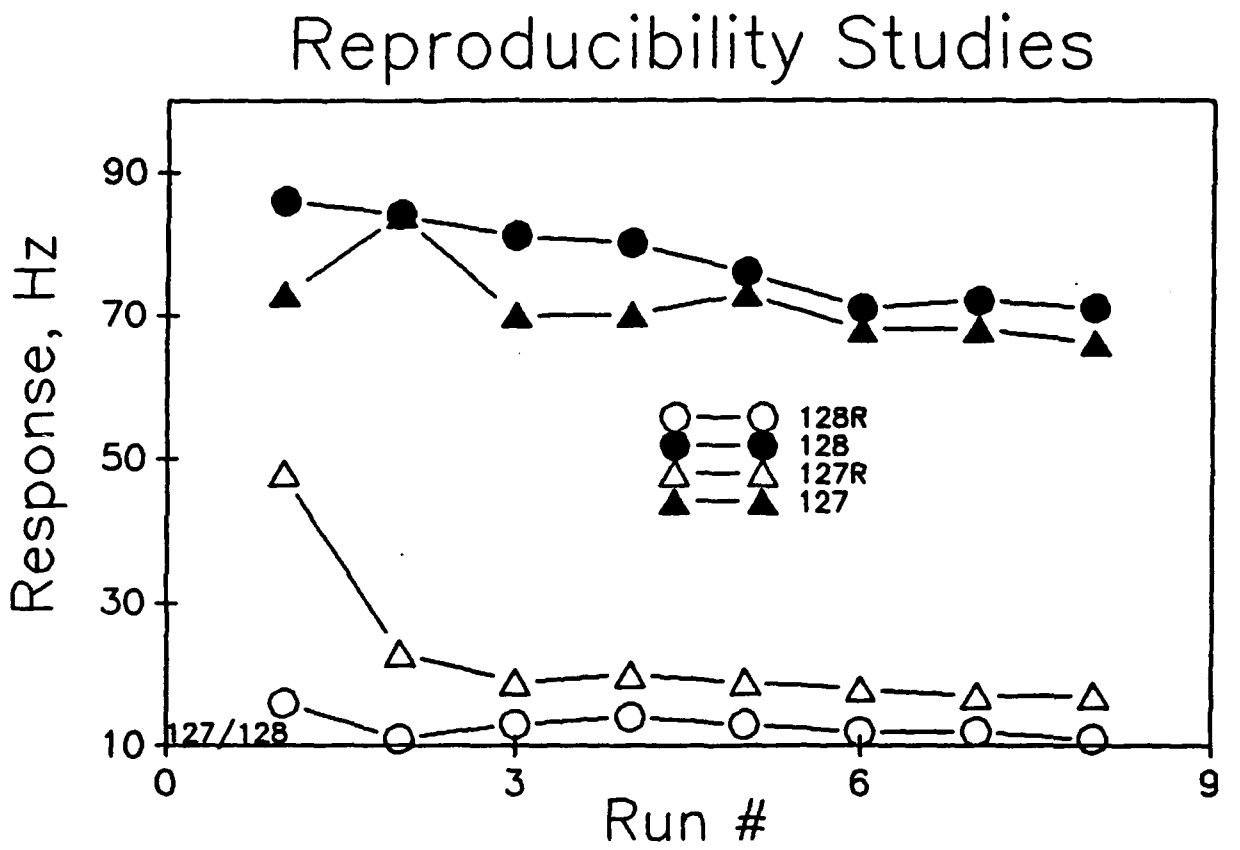

Reproducibility Studies
Response, Hz
90
70
50
30
10
0
3
6
9
Run #
128R
128
127R
127
127/128

TABLE 1

CRYSTAL COATING SOLUTIONS USED

CRYSTAL --- #	WASH	CONCENTRATION OF PROTEIN COATING GELATIN µg/µL	BSA µg/µL	ACH µg/µL	TOTAL AMOUNTS USED GLUT %	VOLUME PER SIDE PROTEIN µL	GLUT µL
1	SB	20.00	0.00	20.00	10.00	0.25	0.03
3	SB	20.00	0.00	20.00	10.00	0.50	0.05
4	SB	20.00	0.00	20.00	10.00	1.00	0.10
5	SB	20.00	0.00	20.00	10.00	1.50	0.15
6	SB	20.00	0.00	20.00	10.00	2.00	0.02
7	SB	20.00	0.00	20.00	10.00	2.50	0.25
8	SB	20.00	0.00	20.00	10.00	3.00	0.30
9R	SB	20.00	0.00	0.00	10.00	3.00	0.30
10	B	10.00	0.00	20.00	10.00	0.15	0.02
10R	B	10.00	0.00	0.00	10.00	0.45	0.04
11	B	10.00	0.00	20.00	10.00	0.25	0.02
11R	B	10.00	0.00	0.00	10.00	0.75	0.07
12	B	10.00	0.00	20.00	10.00	0.40	0.04
12R	B	10.00	0.00	0.00	10.00	1.20	0.12
13	B	10.00	0.00	20.00	10.00	0.50	0.05
13R	B	10.00	0.00	0.00	10.00	1.50	0.15
14	B	10.00	0.00	20.00	10.00	0.70	0.07
14R	B	10.00	0.00	0.00	10.00	2.10	0.20
15	B	14.00	0.00	20.00	5.00	0.50	0.05
15R	B	14.00	0.00	0.00	5.00	1.20	0.12
16	B	14.00	0.00	20.00	5.00	0.75	0.08
16R	B	14.00	0.00	0.00	5.00	1.80	0.18
17	B	14.00	0.00	20.00	5.00	1.25	0.13
17R	B	14.00	0.00	0.00	5.00	1.25	0.13
18	B	14.00	0.00	20.00	5.00	2.00	0.20
18R	B	14.00	0.00	0.00	5.00	2.00	0.20
19	SB	20.00	0.00	20.00	10.00	0.25	0.03
19R	SB	20.00	0.00	0.00	10.00	0.25	0.03
20	SB	20.00	0.00	20.00	10.00	0.50	0.05
20R	SB	20.00	0.00	0.00	10.00	0.50	0.05
21	SWAB	5.00	0.00	50.00	10.00	0.50	0.05
21R	SWAB	10.00	0.00	0.00	10.00	0.50	0.05
22	SWAB	5.00	0.00	50.00	10.00	1.00	0.10
22R	SWAB	10.00	0.00	0.00	10.00	1.00	0.10
23	B	0.00	5.00	12.50	10.00	0.50	0.05
23R	B	0.00	17.50	0.00	10.00	0.50	0.05
24	B	0.00	5.00	12.50	10.00	0.75	0.08
24R	B	0.00	17.50	0.00	10.00	0.75	0.08
25	B	0.00	5.00	12.50	10.00	1.00	0.10
25R	B	0.00	17.50	0.00	10.00	1.00	0.10
26R	B	0.00	17.50	0.00	10.00	1.50	0.15
26	B	0.00	5.00	12.50	10.00	1.50	0.15
27	B	0.00	10.00	25.00	10.00	0.50	0.05

27R	B	0.00	35.00	0.00	10.00	0.50	0.05
28	B	0.00	10.00	25.00	10.00	0.75	0.08
28R	B	0.00	35.00	0.00	10.00	0.75	0.08
29	B	0.00	10.00	25.00	10.00	1.00	0.10
29R	B	0.00	35.00	0.00	10.00	1.00	0.10
30	B	0.00	10.00	25.00	10.00	1.30	0.13
30R	B	0.00	35.00	0.00	10.00	1.30	0.13
31	B	10.00	0.00	25.00	10.00	0.75	0.08
31R	B	35.00	0.00	0.00	10.00	0.75	0.08
32	B	10.00	0.00	25.00	10.00	1.30	0.13
32R	B	35.00	0.00	0.00	10.00	1.30	0.13
33	B	10.00	0.00	25.00	10.00	1.30	0.13
34	B	10.00	0.00	25.00	10.00	0.75	0.08
35	B	0.00	5.00	12.50	10.00	0.50	0.05
36R	B	0.00	17.50	0.00	10.00	0.50	0.05
37	B	0.00	5.00	12.50	10.00	0.75	0.08
38R	B	0.00	17.50	0.00	10.00	0.75	0.08
39	B	0.00	5.00	12.50	10.00	1.00	0.10
40R	B	0.00	17.50	0.00	10.00	1.00	0.10
41R	B	0.00	8.50	0.00	10.00	1.50	0.15
42	B	0.00	2.50	6.00	10.00	1.00	0.10
43	B	0.00	2.50	6.00	10.00	1.50	0.15
41	B	0.00	2.50	6.00	10.00	2.00	0.20
44A	BW	10.00	0.00	25.00	10.00	2.00	0.20
44R	BW	35.00	0.00	0.00	10.00	4.00	0.40
44B	BW	10.00	0.00	25.00	10.00	2.00	0.20
45 - 49 Dea Dextran/Tween 80/cholesterol coated. Non-responsive. No DATA							
50	W	12.50	0.00	50.00	10.00	0.25	0.03
50R	W	12.50	0.00	0.00	10.00	0.31	0.03
51	W	12.50	0.00	50.00	10.00	0.50	0.05
51R	W	12.50	0.00	0.00	10.00	0.63	0.06
52	W	12.50	0.00	50.00	10.00	0.75	0.08
52R	W	12.50	0.00	0.00	10.00	0.92	0.09
53	W	12.50	0.00	50.00	10.00	1.00	0.01
53R	W	12.50	0.00	0.00	10.00	1.30	0.12
54	W	12.50	0.00	50.00	10.00	1.50	0.15
54R	W	12.50	0.00	0.00	10.00	1.89	0.18
55	SW	0.00	5.83	16.67	10.00	1.00	0.10
55R	SW	0.00	22.83	0.00	10.00	1.00	0.10
56	SW	0.00	5.83	16.67	10.00	1.30	0.13
56R	SW	0.00	22.83	0.00	10.00	1.30	0.13
57	SW	0.00	5.83	16.67	10.00	1.70	0.17
57R	SW	0.00	22.83	0.00	10.00	1.70	0.17
58	SW	0.00	5.83	16.67	10.00	2.00	0.20
58R	SW	0.00	22.83	0.00	10.00	2.00	0.20
59	SW	0.00	5.83	16.67	10.00	2.50	0.25
59R	SW	0.00	22.83	0.00	10.00	2.50	0.25
60A		0.00	0.00	0.00	0.00	0.00	0.00
60B		0.00	0.00	0.00	0.00	0.00	0.00
61	W	0.00	25.00	8.75	1.67	1.00	0.20
61R	W	0.00	33.75	0.00	1.67	1.00	0.20
62	W	0.00	25.00	8.75	1.67	1.30	0.20
62R	W	0.00	33.75	0.00	1.67	1.30	0.20
63	W	0.00	25.00	8.75	1.67	1.70	0.34

63R	W	0.00	33.75	0.00	1.67	1.70	0.34
64	W	0.00	25.00	8.75	1.67	2.00	0.40
64R	W	0.00	33.75	0.00	1.67	2.00	0.40
65	W	0.00	25.00	8.75	1.67	2.50	0.50
65R	W	0.00	33.75	0.00	1.67	2.50	0.50

EXPLANATION OF CODES

S - SURFACTANT (SDS)
B - BUFFER
W - DEIONIZED WATER
A - ACETONE
R - REFERENCE

TABLE 2

FINAL CRYSTAL COATINGS USED

CRYSTAL	PROTEIN/CRYSTAL				GLUT	TOTAL WT %	
#	GEL µg	BSA µg	ACH µg	ACH units	GLUT µg	PROTEIN %	GLUT %
1	10.00	0.00	10.00	12.50	6.00	76.92	23.08
3	20.00	0.00	20.00	25.00	10.00	80.00	20.00
4	40.00	0.00	40.00	50.00	20.00	80.00	20.00
5	60.00	0.00	60.00	75.00	30.00	80.00	20.00
6	80.00	0.00	80.00	100.00	40.00	80.00	20.00
7	100.00	0.00	100.00	125.00	50.00	80.00	20.00
8	120.00	0.00	120.00	150.00	60.00	80.00	20.00
9R	120.00	0.00	0.00	0.00	60.00	50.00	50.00
10	3.00	0.00	6.00	7.50	4.00	69.23	30.77
10R	9.00	0.00	0.00	0.00	8.00	52.94	47.06
11	5.00	0.00	10.00	12.50	4.00	78.95	21.05
11R	15.00	0.00	0.00	0.00	14.00	51.72	48.28
12	8.00	0.00	16.00	20.00	8.00	75.00	25.00
12R	24.00	0.00	0.00	0.00	24.00	50.00	50.00
13	10.00	0.00	20.00	25.00	10.00	75.00	25.00
13R	30.00	0.00	0.00	0.00	30.00	50.00	50.00
14	14.00	0.00	28.00	35.00	14.00	75.00	25.00
14R	42.00	0.00	0.00	0.00	40.00	51.22	48.78
15	14.00	0.00	20.00	12.50	5.00	87.18	12.82
15R	33.60	0.00	0.00	0.00	12.00	73.68	26.32
16	21.00	0.00	60.00	75.00	8.00	91.00	9.00
16R	50.96	0.00	0.00	0.00	18.00	73.90	26.10
17	35.00	0.00	100.00	125.00	13.00	91.22	8.78
17R	35.00	0.00	0.00	0.00	13.00	72.92	27.08
18	56.00	0.00	160.00	200.00	20.00	91.52	8.48
18R	56.00	0.00	0.00	0.00	20.00	73.68	26.32
19	10.00	0.00	10.00	12.50	6.00	76.92	23.08
19R	10.00	0.00	0.00	0.00	6.00	62.50	37.50
20	20.00	0.00	20.00	25.00	10.00	80.00	20.00
20R	20.00	0.00	0.00	0.00	10.00	66.67	33.33
21	5.00	0.00	50.00	62.50	10.00	84.61	15.39
21R	10.00	0.00	0.00	0.00	10.00	50.00	50.00
22	10.00	0.00	100.00	125.00	20.00	84.61	15.39
22R	20.00	0.00	0.00	0.00	20.00	50.00	50.00
23	0.00	5.00	12.50	15.63	10.00	63.64	36.36
23R	0.00	17.50	0.00	0.00	10.00	63.64	36.36
24	0.00	7.50	18.75	23.44	16.00	62.13	37.87
24R	0.00	26.25	0.00	0.00	16.00	62.13	37.87
25	0.00	10.00	25.00	31.25	20.00	63.64	36.36
25R	0.00	35.00	0.00	0.00	20.00	63.64	36.36
26	0.00	15.00	37.50	46.88	30.00	63.64	36.36
26R	0.00	52.50	0.00	0.00	30.00	63.64	36.36
27	0.00	10.00	25.00	31.25	10.00	77.78	22.22
27R	0.00	35.00	0.00	0.00	10.00	77.78	22.22

28	0.00	15.00	37.50	46.88	15.00	77.78	22.22
28R	0.00	52.50	0.00	0.00	15.00	77.78	22.22
29	0.00	20.00	50.00	62.50	20.00	77.78	22.22
29R	0.00	70.00	0.00	0.00	20.00	77.78	22.22
30	0.00	26.00	65.00	81.25	26.00	77.78	22.22
30R	0.00	91.00	0.00	0.00	26.00	77.78	22.22
31	15.00	0.00	37.50	46.88	15.00	77.78	22.22
31R	52.50	0.00	0.00	0.00	15.00	77.78	22.22
32	26.00	0.00	65.00	81.25	26.00	77.78	22.22
32R	91.00	0.00	0.00	0.00	26.00	77.78	22.22
33	26.00	0.00	65.00	81.25	26.00	77.78	22.22
34	15.00	0.00	37.50	46.88	15.00	77.78	22.22
35	0.00	5.00	12.50	15.63	10.00	63.64	36.36
36R	0.00	17.50	0.00	0.00	10.00	63.64	36.36
37	0.00	7.50	18.75	23.44	15.00	63.64	36.36
38R	0.00	26.25	0.00	0.00	15.00	63.64	36.36
39	0.00	10.00	25.00	31.25	20.00	63.64	36.36
40R	0.00	35.00	0.00	0.00	20.00	63.64	36.36
41R	0.00	25.50	0.00	0.00	30.00	45.95	54.05
42	0.00	5.00	12.00	15.00	20.00	45.95	54.05
43	0.00	7.50	18.00	22.50	30.00	45.95	54.05
41	0.00	10.00	24.00	30.00	40.00	45.95	54.05
44A	40.00	0.00	100.00	125.00	40.00	77.78	22.22
44R	280.00	0.00	0.00	0.00	80.00	77.78	22.22
44B	40.00	0.00	100.00	125.00	40.00	77.78	22.22
45 - 49 Dea Dextran/Tween 80/cholesterol coated. Non-responsive. No Data.							
50	1.56	0.00	6.25	7.81	5.00	60.97	39.03
50R	7.81	0.00	0.00	0.00	6.00	56.55	43.45
51	3.13	0.00	12.50	15.63	10.00	60.98	39.02
51R	15.80	0.00	0.00	0.00	12.00	56.83	43.17
52	4.69	0.00	18.75	23.44	15.00	60.98	39.02
52R	22.94	0.00	0.00	0.00	18.20	55.76	44.24
53	6.25	0.00	25.00	31.25	20.00	60.98	39.02
53R	31.63	0.00	0.00	0.00	24.00	56.86	43.14
54	9.38	0.00	37.50	46.88	30.00	60.98	39.02
54R	47.25	0.00	0.00	0.00	36.00	56.76	43.24
55	0.00	11.66	33.34	41.68	20.00	69.23	30.77
55R	0.00	45.02	0.00	0.00	20.00	69.24	30.76
56	0.00	15.16	43.34	54.18	26.00	69.23	30.77
56R	0.00	58.50	0.00	0.00	26.00	69.23	30.77
57	0.00	19.82	56.68	70.85	34.00	69.23	30.77
57R	0.00	76.50	0.00	0.00	34.00	69.23	30.77
58	0.00	23.32	66.88	83.35	40.00	69.23	30.77
58R	0.00	90.00	0.00	0.00	40.00	69.23	30.77
59	0.00	29.15	83.35	104.19	50.00	69.23	30.77
59R	0.00	112.50	0.00	0.00	50.00	69.23	30.77
60A							
60B							
61	0.00	50.00	17.50	17.59	6.67	91.01	8.99
61R	0.00	67.50	0.00	0.00	6.67	91.01	8.99
62	0.00	65.00	22.75	22.86	6.67	92.94	7.06
62R	0.00	87.75	0.00	0.00	6.67	92.94	7.06
63	0.00	85.00	29.75	29.90	6.67	91.01	8.99
63R	0.00	114.75	0.00	0.00	6.67	91.01	8.99

64	0.00	100.00	35.00	35.18	13.34	91.01	8.99
64R	0.00	135.00	0.00	0.00	13.34	91.01	8.99
65	0.00	125.00	43.75	43.97	16.67	91.01	8.99
65R	0.00	168.75	0.00	0.00	16.67	91.01	8.99

EXPLANATION OF CODES

R - REFERENCE

TABLE 3

RESPONSES OF COATED CRYSTALS

CRYSTAL	TOTAL COATING/CRYSTAL			RESPONSE*	RESPONSE*
#	µg	Hz	units	Hz	Hz
1	26.00	30401	12.50	491.80	
2					
3	50.00	16051	25.00	247.40	
4	100.00	35252	50.00	276.50	
5	150.00	NO	75.00	NA	
6	200.00	22991	100.00	NR	
7	250.00	25370	125.00	NR	
8	300.00	NO	150.00	NA	
9R	180.00	NO	0.00	NA	
10	13.00	13091	0.75	NR	
10R	17.00	13123	0.00	102.90	
11	19.00	10447	1.25	77.70	
11R	29.00	13763	0.00	102.80	- 25.10
12	32.00	15646	7.00	98.60	
12R	48.00	14110	0.00	106.30	- 7.70
13	40.00	16901	2.50	126.20	
13R	60.00	18204	0.00	142.50	- 16.30
14	56.00	13954	3.50	69.50	
14R	82.00	33449	0.00	201.50	-144.00
15	39.00	27961	12.50	NR	
15R	45.60	NO	0.00	3 OT	
16	89.00	22853	37.50	NR	
16R	68.96	NO	0.00	5 OT	
17	148.00	36048	62.50	NR	
17R	48.00	NO	0.00	5 OT	
18	236.00	NO	100.00	NR	
18R	76.00	NO	0.00	5 OT	
19	26.00	75652	25.00	NR	
19R	16.00	43461	0.00	NR	
20	50.00	NO	50.00	NA	
20R	30.00	25472	0.00	NR	
21	65.00	66140	62.50	55.10	
21R	20.00	6137	0.00	8.40	46.70
22	130.00	76998	125.00	148.10	
22R	40.00	10393	0.00	21.40	126.70
23	27.50	1313	15.63	27.20	
23R	27.50	651	0.00	15.80	11.40
24	42.25	2650	23.44	53.30	
24R	42.25	1606	0.00	29.50	23.80
25	55.00	3060	31.25	58.60	
25R	55.00	2089	0.00	37.50	21.10
26	82.50	5704	46.88	100.80	21.90
26R	82.50	4225	0.00	78.90	
27	45.00	24628	31.25	44.10	

27R	45.00	27453	0.00	65.00	- 20.90
28	67.50	26301	46.88	84.60	
28R	67.50	45018	0.00	110.40	- 25.80
29	90.00	47333	62.50	91.10	
29R	90.00	65513	0.00	118.20	- 27.10
30	117.00	68879	81.25	177.20	
30R	117.00	85140	0.00	NR	
31	67.50	42209	46.88	84.90	
31R	67.50	44337	0.00	82.60	2.30
32	117.00	38403	81.25	90.40	
32R	117.00	54959	0.00	NR	
33	117.00	80974	81.25	98.00	
34	67.50	50531	46.88	NR	
35	27.50	64928	15.63	NR	
36R	27.50		0.00	NA	
37	41.25	51011	23.44	NR	
38R	41.25	92154	0.00	NR	
39	55.00	17003	31.25	122.20	
40R	55.00		0.00	NO	
41R	55.50	33630	0.00	NO	
42	37.00	83720	15.00	32.90	
43	55.50	61995	22.50	46.90	
41	74.00	45135	30.00	68.20	
44A	180.00	32111	125.00	77.10	
44R	360.00	52700	0.00	150.15	- 73.05
44B	180.00	37718	125.00	92.10	- 58.05
45 - 49 DEAE Dextran/Tween 80/cholesterol coated. Non-responsive. No Data					
50	12.81	1786	7.81	18.00	
50R	13.81	2290	0.00	16.80	2.80
51	25.63	9733	15.63	38.93	
51R	27.80	10297	0.00	38.40	0.53
52	38.44	11654	23.44	38.70	
52R	41.14	4404	0.00	71.25	- 32.55
53	51.25	19017	31.25	59.65	
53R	55.63	- 9176	0.00	57.35	2.30
54	76.88	16223	46.88	43.58	
54R	83.25	-18919	0.00	38.02	5.56
55	45.02	46303	41.68	176.16	
55R	45.02	55543	0.00	215.83	- 39.67
56	58.51	43148	54.18	156.60	
56R	58.51	-26318	0.00	1 OT	
57	76.51	-26912	70.85	1 OT	
57R	76.51	38450	0.00	148.20	
58	90.04	91187	83.35	292.25	
58R	90.04	NO	0.00	NO	
59	112.55	27770	104.19	183.60	
59R	112.55	NO	0.00	NO	
60A				28.40	
60B				28.70	
61	74.17	-28702	17.59		
61R	74.17	NO	0.00		
62	94.42	16740	22.86		
62R	94.42	NO	0.00		
63	126.09	NO	0.00		

63R	126.09	NO	0.00
64	148.45	NO	35.18
64R	148.34	NO	0.00
65	185.42	NO	43.97
65R	185.42	NO	0.00

EXPLANATION OF CODES

R - REFERENCE
NO - NOT OSCILLATING
NA - NOT APPLICABLE
NR - OSCILLATING, BUT NO RESPONSE AFTER SAMPLE INTRODUCTION
OT - OVERTONE

TABLE 4

REPRESENTATIVE RESPONSES* OF

ENZYME/BSA/GLUTARALDEHYDE/SURFACTANT COATED CRYSTALS

ENZYME	BSA	GA (0.1%)	SURFACTANT	RESPONSE
units	mg	μL		Hz
5.60	0.00	0.00	Tween	27.00
5.60	0.00	0.00	Triton	66.00
31.00	0.07	0.50	Tween	5.00
31.00	0.07	0.50	Triton	329.00
31.00	0.07	0.00	Tween	312.00
31.00	0.07	0.00	Triton	648.00
31.00	0.07	0.50	None	1241.00
31.00	0.00	0.50	Tergitol	289.00
31.00	0.00	0.50	SDS	254.00

Flow Rate = 100 mL/min
Response* = response to DMMP
Generation method = Bubbler

TABLE 5

REPRESENTATIVE RESPONSES[a] OF
ENZYME/BSA/GLUTARALDEHYDE COATED CRYSTALS

ENZYME	BSA	GLUTARALDEHYDE (0.1%)	RESPONSE
units	mg	μL	Hz
4.00	0.00	0.00	28.00
5.60	0.00	0.00	35.00
4.00	0.00	0.20	23.00
14.00	0.00	0.00	30.00
14.00	0.00	0.00	104.00
4.00	0.00	0.20	30.00
5.60	0.00	0.00	35.00
5.60	0.00	0.50	68.00
31.00	0.07	0.50	1241.00
31.00	0.07	0.20	804.00
31.00	0.07	1.00	376.00
0.00	0.07	0.00	6.00

Flow Rate = 100 mL/min
Response[a] = response to DMMP
Generation method = Bubbler

TABLE 6

REPRESENTATIVE RESPONSES OF
ENZYME/BSA/GLUTARALDEHYDE/SURFACTANT COATED CRYSTALS

ENZYME	BSA	GA (0.1%)	SURFACTANT	RESPONSE
units	mg	μL		Hz
82.00	0.10	0.00	Triton	10.00
41.00	0.50	0.50	None	135.00
82.00	0.00	0.50	None	102.00
82.00	0.00	0.75	None	135.00
82.00	0.00	0.25	None	128.00
24.00	0.00	0.20	None	82.00
24.00	0.00	0.20	None	132.00
24.00	0.00	0.10	None	108.00
20.00	0.08	0.50	Triton	181.00
20.00	0.08	0.50	None	160.00
30.00	0.12	0.50	None	189.00
40.00	0.16	0.50	None	189.00
20.00	0.00	0.50	None	4.00
40.00	0.16	0.00	Triton	40.00
20.00	0.00	0.20	Triton	60.00

Flow rate = 100 mL/min
[DMMP] = 0.90 ppm
Generation method = Gas permeaton tube

TABLE 7

RESULTS OBTAINED USING BSA COATED REFERENCE CRYSTALS

CRYSTAL	BSA+ENZ[a]	BSA	GA(0.1%)	RESPONSE[b]	REFERENCE[c]
#	µL	mg	µL	Hz	
1	0.00	0.14	0.20	2.00	BLANK
10	0.00	0.14	0.50	67.00	BLANK
12	1.00	0.00	0.50	101.00	BLANK
				12.00	#10
13	1.00	0.00	0.50	28.00	#10
17	2.00	0.00	0.50	75.00	#10
19	1.00	0.00	0.20	9.00	#10
22	1.00	0.00	0.20	145.00	BLANK
				53.00	#10

[a] Each µl of this solution contains 130 enzyme units and 0.14 mg BSA

[b] 0.9 ppm DMMP; flow rate = 100mL/min

TABLE 8

EFFECT OF THE TOTAL WEIGHT OF PROTEIN COATING

CRYSTAL #	ENZYME μL	BSA mg	GA(0.1%) μL	RESPONSE[a] Hz	COMMENTS[b]
2	13.00	0.060	0.50	47.00	ref. #22
22	0.00	0.080	0.50	0.00	
10	19.00	0.090	0.50	31.00	
54	13.00	0.060	0.50	68.00	
200	13.00	0.060	0.30	4.00	
7	21.00	0.096	0.50	155.00	
17	21.00	0.136	0.50	102.00	
8	31.00	0.104	0.50	18.00	
16	15.00	0.132	0.50	- 10.00	negative
21	21.00	0.024	0.75	- 16.00	negative
54	16.00	0.132	0.75	30.00	
13	11.00	0.088	0.50	277.00	
9	8.00	0.086	0.50	256.00	
11	30.00	0.120	0.50	234.00	

[a] Response to DMMP
Flow rate = 100/mL/min

[b] Negative - reference crystal gave a higher response than active crystal

TABLE 9

EFFECT OF THE EXPOSURE TIME ON RESPONSE OF A CHOLINESTERASE COATED CRYSTAL TO DMMP (0.9 ppm)

% TOTAL RESPONSE* in Hz			
RUN #	EXPOSURE TIME TO DMMP		
	30 sec	60 sec	90 sec
1	46	71	89
2	43	70	85
3	44	74	86
4	48	73	88
5	45	72	87

TABLE 10

Study of the Inhibition of Cholinesterase in the PZ Crystal by DMMP

Crystal	Treatment	Absorbance
1	No exp to DMMP	0.502
2	No exp to DMMP	0.48
3	6 min to DMMP	0.525
4	15 min to DMMP	0.500
5	No exp to DMMP	0.48
6	Heat Deactivation	0.542
7	6 min exp to DMMP	0.535
8	15 min to DMMP	0.473

TABLE 11

RESPONSE OF SELECTED BSA COATED CRYSTALS TO DMMP

CRYSTAL #	ENZYME mg	BSA mg	GA(0.1%) µL	RESPONSE* Hz	REFERENCE
8	0.00750	0.00750	0.50	22.00	9
9	0.00	0.01500	0.50	- 55.00	11
9				59.00	22
9				- 4.00	13
9				52.00	16
11	0.00750	0.00750	0.50	21.00	12
12	0.00750	0.00750	0.00		
13	0.00	0.15000	0.00	4.00	9
16	0.11250	0.00	0.50	- 34.00	17
17	0.00	0.11250	0.50	27.00	52
21		0.00750	0.00		
22	0.01500	0.00	0.50	16.00	51R
51R	0.01875	0.00	0.50		
52	0.00	0.02250			

* Response to 0.9 ppm DMMP
Flow rate = 100 mL/min

TABLE 12

RESPONSE OF SELECTED BSA COATED CRYSTALS TO DMMP

CRYSTAL #	ENZYME µ	ENZYME mg	BSA mg	DEXTRAN mg	SUCROSE mg	GA(0.1%) µL	RESPONSE* Hz	REFERENCE
1	2.0	0.008	0.040	0.00	0.00	0.00	191.00	3'
1							20.00	3
2	2.0	0.008	0.040	0.00	0.00	0.50	20.00	4
3'	0.0	0.00	0.048	0.00	0.00	0.50	342.00	3
3	8.4	0.008	0.008	0.00	0.00	0.50 (1%)		
4	0.0	0.00	0.048	0.00	0.00	0.50	7.00	16
4							350.00	52R
5	8.4	0.008	0.040	0.00	0.00	0.00	36.00	3
6	8.4	0.008	0.040	0.00	0.00	0.50		
8	8.4	0.008	0.040	0.00	0.00	0.50 (5%)	307.00	3
16	2.0	0.008	0.00	0.008	0.008	0.50	55.00	17
17	0.0	0.00	0.008	0.008	0.008	0.50	10.00	18
21	8.4	0.008	0.040	0.00	0.00	0.50 (1%)	6.00	4
50	0.0	0.00	0.048	0.00	0.00	0.50 (1%)	21.00	3
52	8.4	0.00	0.00	0.016	0.00	0.50	10.00	52R
52R	0.0	0.00	0.008	0.008	0.00	0.50		
54	0.0	0.00	0.008	0.008	0.00	0.50 (1%)		
13	0.0	0.00	0.040	0.008	0.00	0.50	290.00	52
13							407.00	52R

* Response to DMMP 0.9 ppm
Flow rate = 100 mL/min

TABLE 13

RESPONSES OF SELECTED BSA COATED CRYSTALS TO DMMP

CRYSTAL #	ENZYME mg	BSA mg	DEXTRAN mg	GA(0.1%) µL	RESPONSE* Hz	REFERENCE
1	0.008	0.04	0.00	0.50	34.00	8
8	0.00	0.048	0.00	0.50	41.00	13
9	0.016	0.00	0.00	0.50	7.00	10
9					- 66.00	54
10	0.00	0.016	0.00	0.50		
13	0.008	0.008	0.00	0.50	-214.00	17
66	0.00	0.088	0.00	0.50	39.00	13
66					16.00	8
17	0.00	0.056	0.00	0.50	190.00	8
21	0.024	0.00	0.00	0.00	18.00	22
22	0.00	0.024	0.00	0.00	11.00	50
50	0.032	0.00	0.00	0.00		
51R	0.00	0.008	0.04	0.00	-104.00	8
52R	0.00	0.048	0.00	0.00	117.00	52
52	0.008	0.040	0.00	0.00		
54	0.00	0.00	0.04	0.50		
54R	0.00	0.040	0.00	0.50	310.00	54
100	0.00	0.00	0.04			
112	0.00	0.04	0.00	0.00	132.00	100

* Response to DMMP 0.9 ppm
Flow Rate = 100 mL/min

TABLE 14

CRYSTAL IMMOBILIZATION SOLUTIONS

CRYSTAL #	WASH	CONCENTRATION OF PROTEIN GELATIN mg/mL	BSA mg/mL	GD-1 mg/mL	GLUT %	TOTAL VOLUME PER SIDE PROTEIN µL	GLUT µL
66		0.00	0.00	3.40	0.00	1.00	0.00
66R		0.00	3.40	0.00	0.00	1.00	0.00
67		0.00	0.00	3.40	0.00	1.20	0.00
67R		0.00	3.40	0.00	0.00	1.20	0.00
68		0.00	0.00	3.40	0.00	1.60	0.00
68R		0.00	3.40	0.00	0.00	1.60	0.00
69		0.00	0.00	3.40	0.00	1.80	0.00
69R		0.00	3.40	0.00	0.00	1.80	0.00
70		0.00	0.00	3.40	10.00	1.00	0.10
70R		0.00	3.40	0.00	10.00	1.00	0.10
71		0.00	0.00	3.40	10.00	1.20	0.12
71R		0.00	3.40	0.00	10.00	1.20	0.12
72		0.00	0.00	3.40	10.00	1.60	0.16
72R		0.00	3.40	0.00	10.00	1.60	0.16
73		0.00	0.00	3.40	10.00	1.80	0.18
73R		0.00	3.40	0.00	10.00	1.80	0.18
74		0.00	1.70	1.70	10.00	1.00	0.10
74R		0.00	3.40	0.00	10.00	1.00	0.10
75		0.00	1.70	1.70	10.00	1.20	0.12
75R		0.00	3.40	0.00	10.00	1.20	0.16
76		0.00	1.70	1.70	10.00	1.60	0.16
76R		0.00	3.40	0.00	10.00	1.60	0.18
77		0.00	1.70	1.70	10.00	1.80	0.18
77R		0.00	3.40	0.00	10.00	1.80	0.18
78		0.00	0.00	3.40	10.00	1.00	0.10
78R		3.40	0.00	0.00	10.00	1.00	0.10
79		0.00	0.00	3.40	10.00	1.20	0.12
79R		3.40	0.00	0.00	10.00	1.20	0.12
80		0.00	0.00	3.40	10.00	1.60	0.16
80R		3.40	0.00	0.00	10.00	1.60	0.16
81		0.00	0.00	3.40	10.00	1.80	0.18
81R		3.40	0.00	0.00	10.00	1.80	0.18
82		0.00	0.00	3.40	10.00	1.20	0.12
82R		0.00	3.40	0.00	10.00	1.20	0.12
83R		0.00	3.40	0.00	10.00	1.60	0.16
84		0.00	0.00	3.40	10.00	1.00	0.10
84R		0.00	3.40	0.00	10.00	1.00	0.10
85		0.00	0.00	3.40	10.00	1.40	0.14
85R		0.00	3.40	0.00	10.00	1.40	0.14
86		0.00	0.00	3.40	10.00	1.80	0.18
86R		0.00	3.40	0.00	10.00	1.80	0.18
87		0.00	0.00	3.40	0.00	1.00	0.00
87R		0.00	3.40	0.00	0.00	1.00	0.00

88	0.00	0.00	3.40	0.00	1.20	0.00
88R	0.00	3.40	0.00	0.00	1.20	0.00
89	0.00	0.00	3.40	0.00	1.60	0.00
89R	0.00	3.40	0.00	0.00	1.60	0.00
111	0.00	0.00	3.40	3.40	1.00	0.10
111R	0.00	3.40	0.00	3.40	1.00	0.10
112	0.00	0.00	3.40	3.40	1.50	0.15
112R	0.00	3.40	0.00	3.40	1.50	0.15
113	0.00	0.00	3.40	0.00	1.00	0.00
113R	0.00	3.40	0.00	0.00	1.00	0.00
114	0.00	0.00	3.40	0.34	1.00	0.10
114R	0.00	3.40	0.00	0.34	1.00	0.10
115	0.00	0.00	3.40	0.34	1.00	0.10
115R	0.00	3.40	0.00	0.34	1.00	0.10
123	0.00	0.00	3.40	0.175	1.20	0.12
123R	0.00	10.00	0.00	0.175	1.20	0.12
124	0.00	0.00	3.40	0.175	1.40	0.14
124R	0.00	10.00	0.00	0.175	1.40	0.14
125	0.00	0.00	3.40	0.175	1.60	0.16
125R	0.00	10.00	0.00	0.175	1.60	0.16
126	0.00	0.00	3.40	0.175	1.80	0.18
126R	0.00	10.00	0.00	0.175	1.80	0.18

EXPLANATION OF CODES

S - SURFACTANT (SDS)
B - BUFFER
W - DEIONIZED WATER
A - ACETONE
R - REFERENCE
GD-1 - Anti Soman Antibody

TABLE 15

FINAL CRYSTAL COATINGS USED

CRYSTAL	PROTEIN/CRYSTAL RATIO				GLUT	TOTAL WT %	
#	GEL mg	BSA mg	GD-1 mg	GD-1 dil factor	GLUT mg	PROTEIN %	GLUT %
66	0.00	0.00	6.80	0.00	0.00	100.00	0.00
66R	0.00	6.80	0.00	0.00	0.00	100.00	0.00
67	0.00	0.00	8.16	0.00	0.00	100.00	0.00
67R	0.00	8.16	0.00	0.00	0.00	100.00	0.00
68	0.00	0.00	10.88	0.00	0.00	100.00	0.00
68R	0.00	10.88	0.00	0.00	0.00	100.00	0.00
69	0.00	0.00	12.24	0.00	0.00	100.00	0.00
69R	0.00	12.24	0.00	0.00	0.00	100.00	0.00
70	0.00	0.00	6.80	0.00	20.00	25.37	74.63
70R	0.00	6.80	0.00	0.00	20.00	25.37	74.63
71	0.00	0.00	8.16	0.00	24.00	25.37	74.63
71R	0.00	8.16	0.00	0.00	24.00	25.37	74.63
72	0.00	0.00	10.88	0.00	32.00	25.37	74.63
72R	0.00	10.88	0.00	0.00	32.00	25.37	74.63
73	0.00	0.00	12.24	0.00	36.00	25.37	74.63
73R	0.00	12.24	0.00	0.00	36.00	25.37	74.63
74	0.00	3.40	3.40	1:1	20.00	25.37	74.63
74R	0.00	6.80	0.00	1:1	20.00	25.37	74.63
75	0.00	4.08	4.08	1:1	24.00	25.37	74.63
75R	0.00	8.16	0.00	1:1	24.00	25.37	74.63
76	0.00	5.44	5.44	1:1	32.00	25.37	74.63
76R	0.00	10.88	0.00	1:1	32.00	25.37	74.63
77	0.00	6.12	6.12	1:1	36.00	25.37	74.63
77R	0.00	12.24	0.00	1:1	36.00	25.37	74.63
78	0.00	0.00	6.80	0.00	20.00	25.37	74.63
78R	6.80	0.00	0.00	0.00	20.00	25.37	74.63
79	0.00	0.00	8.16	0.00	24.00	25.37	74.63
79R	8.16	0.00	0.00	0.00	24.00	25.37	74.63
80	0.00	0.00	10.88	0.00	32.00	25.37	74.63
80R	10.88	0.00	0.00	0.00	32.00	25.37	74.63
81	0.00	0.00	12.24	0.00	36.00	25.37	74.63
81R	12.24	0.00	0.00	0.00	36.00	25.37	74.63
82	0.00	0.00	8.16	0.00	24.00	25.37	74.63
82R	0.00	8.16	0.00	0.00	24.00	25.37	74.63
83R	0.00	10.88	0.00	0.00	32.00	25.37	74.63
84	0.00	0.00	6.80	0.00	20.00	25.37	74.63
84R	0.00	6.80	0.00	0.00	20.00	25.37	74.63
85	0.00	0.00	9.52	0.00	28.00	25.37	74.63
85R	0.00	9.52	0.00	0.00	28.00	25.37	74.63
86	0.00	0.00	12.24	0.00	36.00	25.37	74.63
86R	0.00	12.24	0.00	0.00	36.00	25.37	74.63
87	0.00	0.00	6.80	0.00	0.00	100.00	0.00
87R	0.00	6.80	0.00	0.00	0.00	100.00	0.00

88	0.00	0.00	8.16	0.00	0.00	100.00	0.00
88R	0.00	8.16	0.00	0.00	0.00	100.00	0.00
89	0.00	0.00	10.88	0.00	0.00	100.00	0.00
89R	0.00	10.88	0.00	0.00	0.00	100.00	0.00
111	0.00	0.00	6.80	0.00	0.68	90.91	9.09
111R	0.00	6.80	0.00	0.00	0.68	90.91	9.09
112	0.00	0.00	10.20	0.00	0.102	90.91	9.09
112R	0.00	10.20	0.00	0.00	0.102	90.91	9.09
113	0.00	0.00	6.80	0.00	0.00	100.00	0.00
113R	0.00	6.80	0.00	0.00	0.00	100.00	0.00
114	0.00	0.00	6.80	0.00	0.34	95.24	4.76
114R	0.00	6.80	0.00	0.00	0.34	95.24	4.76
115	0.00	0.00	6.80	0.00	0.68	90.91	9.09
115R	0.00	6.80	0.00	0.00	0.68	90.91	9.09
123	0.00	0.00	8.16	0.00	0.42	95.10	4.90
123R	0.00	24.00	0.00	0.00	0.42	98.28	1.72
124	0.00	0.00	9.52	0.00	0.49	95.10	4.90
124R	0.00	28.00	0.00	0.00	0.49	98.28	1.72
125	0.00	0.00	10.88	0.00	0.56	95.10	4.90
125R	0.00	32.00	0.00	0.00	0.56	98.28	1.72
126	0.00	0.00	12.24	0.00	0.63	95.10	4.90
126R	0.00	36.00	0.00	0.00	0.63	98.28	1.72

EXPLANATION OF CODES

R - REFERENCE
NO - NOT OSCILLATING
NA - NOT APPLICABLE
NR - OSCILLATING, BUT NO RESPONSE AFTER SAMPLE INTRODUCTION
OT - OVERTONE

TABLE 16

RESPONSE OF ANTIBODY COATED CRYSTALS TO DMMP

CRYSTAL	TOTAL COATING/CRYSTAL		RESPONSE[a]	Ra - Rr[b]
#	µg	Hz	Hz	Hz
66	6.80	17991	146.04	
66R	6.80	3009	48.77	97.27
67	8.16	19655	146.80	
67R	8.16	28398	68.75	78.05
68	10.88	33352	188.47	
68R	10.88	27164	54.53	133.94
69	12.24	36154	198.62	
69R	12.24	28857	44.97	153.65
70	26.80	11683	142.13	
70R	26.80	13536	NR	
71	32.16	16985	217.73	
71R	32.16	14834	NR	
72	42.88	13803	157.43	
72R	42.88	19902	89.46	67.97
73	48.24	16797	135.80	
73R	48.24	14642	99.99	35.81
74	26.80	18021	129.63	
74R	26.80	NA	NR	
75	32.16	19798	140.10	
75R	32.16	52856	102.36	37.74
76	42.88	27324	360.04	
76R	42.88	56188	109.12	250.92
77	48.24	35754	191.31	
77R	48.24	61948	67.58	123.73
78	26.80	20274	132.30	
78R	26.80	6392	58.50	73.80
79	32.16	20090	192.90	
79R	32.16	NO		
80	42.88	29647	190.95	
80R	42.88	16212	75.90	115.05
81	48.24	31332	187.30	
81R	48.24	13488	76.00	111.30
82	32.16	16657	181.60	
82R	32.16	4664	67.70	113.90
83R	42.88	- 9172	88.30	
84	26.80	21717		
84R	26.80	26406		
85	37.52	33244		
85R	37.52	26662		
86	48.24	31157		
86R	48.24	NO		
87	6.80	17200	102.06	
87R	6.80	17399	20.80	81.26
88	8.16	21073	126.03	

88R	8.16	18149	17.21	108.82
89	10.88	25800	118.09	
89R	10.88	20492	15.70	102.39
111	7.48	15320	23.55	
111R	7.48	48467	99.00	- 74.54
112	10.30	18991	24.02	
112R	10.30	40767	86.60	- 62.58
113	6.80	24608	56.75	
113R	6.80	NO	NR	
114	7.14	29022	29.09	
114R	7.14	23636	12.51	16.58
115	7.48	38575	76.45	
115R	7.48	28523	17.34	59.11
116	21.60	3333	12.10	
123	8.58	17519	NO	
123R	24.42	18848	9.20	
124	10.01	16911	15.00	
124R	28.49	21089	19.60	- 4.60
125	11.44	20611	16.40	
125R	32.56	23147	28.40	- 12.00
126	12.87	21176	16.60	
126R	36.63	21232	23.40	- 6.80

EXPLANATION OF CODES

R - REFERENCE
NO - NOT OSCILLATING
NA - NOT APPLICABLE
NR - OSCILLATING, BUT NO RESPONSE AFTER SAMPLE INTRODUCTION
OT - OVERTONE
Ra - ACTIVE RESPONSE
Rr - REFERENCE RESPONSE

* Response to DMMP 0.9 ppm
  Flow rate = 100 mL/min

▷ Active response - reference response

TABLE 17

CRYSTAL COATINGS USED IN anti-DMMP STUDY

CRYSTAL #	WASH	CONCENTRATION OF PROTEIN GELATIN mg/mL	BSA mg/mL	aDMMP mg/mL	GLUT %	TOTAL VOLUME PER SIDE PROTEIN µL	GLUT µL
90		0.00	0.00	3.40	0.00	1.80	0.00
90R		0.00	3.40	0.00	0.00	1.80	0.00
91		0.00	0.00	10.00	0.00	1.00	0.00
91R		0.00	10.00	0.00	0.00	1.00	0.00
92		0.00	0.00	10.00	0.00	1.00	0.00
92R		0.00	10.00	0.00	0.00	1.00	0.00
93		0.00	0.00	10.00	0.00	1.00	0.00
93R		0.00	10.00	0.00	0.00	1.00	0.00
94		0.00	0.00	10.00	0.00	1.00	0.00
94R		0.00	10.00	0.00	0.00	1.00	0.00
95		0.00	0.00	10.00	0.00	1.00	0.00
95R		0.00	10.00	0.00	0.00	1.00	0.00
96		0.00	0.00	10.00	10.00	1.00	0.10
96R		0.00	10.00	0.00	10.00	1.00	0.10
97		0.00	0.00	10.00	10.00	1.20	0.12
97R		0.00	10.00	0.00	10.00	1.20	0.12
98		0.00	0.00	10.00	10.00	1.60	0.16
98R		0.00	10.00	0.00	10.00	1.60	0.16
99		0.00	0.00	10.00	10.00	1.80	0.18
99R		0.00	10.00	0.00	10.00	1.80	0.18
100		0.00	0.00	10.00	10.00	0.40	0.04
100R		0.00	10.00	0.00	10.00	0.40	0.04
101		0.00	0.00	10.00	10.00	0.60	0.06
101R		0.00	10.00	0.00	10.00	0.60	0.06
102		0.00	0.00	10.00	10.00	0.80	0.08
102R		0.00	10.00	0.00	10.00	0.80	0.08
103		0.00	0.00	10.00	10.00	1.00	0.10
103R		0.00	10.00	0.00	10.00	1.00	0.10
104		0.00	0.00	10.00	10.00	1.00	0.10
104R		0.00	10.00	0.00	10.00	1.00	0.10
105		0.00	0.00	10.00	10.00	1.40	0.14
105R		0.00	10.00	0.00	10.00	1.40	0.14
106		0.00	0.00	10.00	10.00	1.60	0.16
106R		0.00	10.00	0.00	10.00	1.60	0.16
107		0.00	0.00	10.00	10.00	1.80	0.18
107R		0.00	10.00	0.00	10.00	1.80	0.18
108A		0.00	0.00	3.40	10.00	1.00	0.10
108B		0.00	0.00	3.40	10.00	1.50	0.15
109A		0.00	0.00	3.40	10.00	1.00	0.10
109B		0.00	0.00	3.40	10.00	1.50	0.15
110A		0.00	3.40	0.00	10.00	1.00	0.10
110B		0.00	3.40	0.00	10.00	1.50	0.15
116	W	0.00	0.00	10.00	0.35	0.80	0.80

116R	W	0.00	10.00	0.00	0.35	0.80	0.80
117		0.00	0.00	10.00	0.35	1.00	0.10
117R	W	0.00	10.00	0.00	0.35	1.00	0.10
118		0.00	0.00	10.00	0.35	1.20	0.12
118R	W	0.00	10.00	0.00	0.35	1.20	0.12
119	W	0.00	0.00	10.00	0.35	1.40	0.14
119R	W	0.00	10.00	0.00	0.35	1.40	0.14
120		0.00	0.00	10.00	0.0175	1.20	0.12
120R		0.00	10.00	0.00	0.0175	1.20	0.12
121		0.00	0.00	10.00	0.0175	1.40	0.14
121R		0.00	10.00	0.00	0.0175	1.40	0.14
122		0.00	0.00	10.00	0.0175	1.60	0.16
122R		0.00	10.00	0.00	0.0175	1.60	0.16
127		0.00	0.00	10.00	0.50	1.20	0.12
127R		0.00	10.00	0.00	0.50	1.20	0.12
128		0.00	0.00	10.00	0.50	1.40	0.14
128R		0.00	10.00	0.00	0.50	1.40	0.14
129		0.00	0.00	10.00	0.50	1.60	0.16
129R		0.00	10.00	0.00	0.50	1.60	0.16
130		0.00	0.00	10.00	0.50	1.80	0.18
130R		0.00	10.00	0.00	0.50	1.80	0.18
131		0.00	0.00	10.00	0.70	1.20	0.12
131R		0.00	10.00	0.00	0.70	1.20	0.12
132		0.00	0.00	10.00	0.70	1.40	0.14
132R		0.00	10.00	0.00	0.70	1.40	0.14
133		0.00	0.00	10.00	0.70	1.60	0.16
133R		0.00	10.00	0.00	0.70	1.60	0.16
134		0.00	0.00	10.00	0.70	1.80	0.18
134R		0.00	10.00	0.00	0.70	1.80	0.18
135		0.00	0.00	32.00	0.70	1.20	0.12
135R		0.00	10.00	0.00	0.70	1.20	0.12
136		0.00	0.00	32.00	0.70	1.40	0.14
136R		0.00	10.00	0.00	0.70	1.40	0.14
137		0.00	0.00	32.00	0.70	1.60	0.16
137R		0.00	10.00	0.00	0.70	1.60	0.16
138		0.00	0.00	32.00	0.70	1.80	0.18
138R		0.00	10.00	0.00	0.70	1.80	0.18
139		0.00	0.00	32.00	0.50	1.50	0.15
139R		0.00	10.00	0.00	0.50	1.50	0.15
140		0.00	0.00	32.00	0.50	1.50	0.15
140R		0.00	10.00	0.00	0.50	1.50	0.15
141		0.00	0.00	5.00	0.25	1.00	0.10
141R		0.00	5.00	0.00	0.25	1.00	0.10
142		0.00	0.00	5.00	0.25	1.00	0.10
142R		0.00	5.00	0.00	0.25	1.00	0.10
143		0.00	0.00	10.00	0.50	1.20	0.12
143R		0.00	10.00	0.00	0.50	1.20	0.12
144		0.00	0.00	10.00	0.50	1.40	0.14
144R		0.00	10.00	0.00	0.50	1.40	0.14
145		0.00	0.00	10.00	0.50	1.60	0.16
145R		0.00	10.00	0.00	0.50	1.60	0.16
146		0.00	0.00	10.00	0.50	1.80	0.18
146R		0.00	10.00	0.00	0.50	1.80	0.18
147		0.00	0.00	10.00	0.50	1.40	0.14

147R	0.00	10.00	0.00	0.50	1.40	0.14
148	0.00	0.00	10.00	0.50	1.60	0.16
148R	0.00	10.00	0.00	0.50	1.60	0.16
149	0.00	0.00	3.20	0.15	1.20	0.12
149R	0.00	3.20	0.00	0.15	1.20	0.12
150	0.00	0.00	3.20	0.15	1.40	0.14
150R	0.00	3.20	0.00	0.15	1.40	0.14
151	0.00	0.00	3.20	0.15	1.60	0.16
151R	0.00	3.20	0.00	0.15	1.60	0.16
152	0.00	0.00	3.20	0.15	1.80	0.18
152R	0.00	3.20	0.00	0.15	1.80	0.18
153	0.00	0.00	0.00	0.00	0.00	0.00
154	0.00	0.00	76.80	0.00	1.20	0.00
154R	0.00	20.00	0.00	0.00	1.20	0.00
155	0.00	0.00	89.60	0.00	1.40	0.00
155R	0.00	20.00	0.00	0.00	1.40	0.00
156	0.00	0.00	102.40	0.00	1.60	0.00
156R	0.00	20.00	0.00	0.00	1.60	0.00
157	0.00	0.00	115.20	0.00	1.80	0.00
158	0.00	0.00	64.00	0.00	1.00	0.00
159	0.00	0.00	20.00	0.00	1.00	0.00

EXPLANATION OF CODES

S - SURFACTANT (SDS)
B - BUFFER
W - DEIONIZED WATER
A - ACETONE
R - REFERENCE
GD-1 - Anti Soman Antibody

TABLE 18

FINAL CRYSTAL COATINGS USED IN THE anti-DMMP STUDY

CRYSTAL	PROTEIN/CRYSTAL				GLUT	TOTAL WT %	
#	GEL mg	BSA mg	aDMMP mg	aDMMP dil factor	GLUT mg	PROTEIN %	GLUT %
90	0.00	0.00	12.24	0.00	0.00	100.00	0.00
90R	0.00	12.24	0.00	0.00	0.00	100.00	0.00
91	0.00	0.00	2.00	1:10	0.00	100.00	0.00
91R	0.00	2.00	0.00	1:10	0.00	100.00	0.00
92	0.00	0.00	0.20	$1:10^{+1}$	0.00	100.00	0.00
92R	0.00	0.20	0.00	$1:10^{+1}$	0.00	100.00	0.00
93	0.00	0.00	0.02	$1:10^{+2}$	0.00	100.00	0.00
93R	0.00	0.02	0.00	$1:10^{+2}$	0.00	100.00	0.00
94	0.00	0.00	0.002	$1:10^{+3}$	0.00	100.00	0.00
94R	0.00	0.002	0.00	$1:10^{+3}$	0.00	100.00	0.00
95	0.00	0.00	0.0002	$1:10^{+4}$	0.00	100.00	0.00
95R	0.00	0.0002	0.00	$1:10^{+4}$	0.00	100.00	0.00
96	0.00	0.00	20.00	0.00	20.00	50.00	50.00
96R	0.00	20.00	0.00	0.00	20.00	50.00	50.00
97	0.00	0.00	24.00	0.00	24.00	50.00	50.00
97R	0.00	24.00	0.00	0.00	24.00	50.00	50.00
98	0.00	0.00	32.00	0.00	32.00	50.00	50.00
98R	0.00	32.00	0.00	0.00	32.00	50.00	50.00
99	0.00	0.00	36.00	0.00	36.00	50.00	50.00
99R	0.00	36.00	0.00	0.00	36.00	50.00	50.00
100	0.00	0.00	8.00	0.00	8.00	50.00	50.00
100R	0.00	8.00	0.00	0.00	8.00	50.00	50.00
101	0.00	0.00	12.00	0.00	12.00	50.00	50.00
101R	0.00	12.00	0.00	0.00	12.00	50.00	50.00
102	0.00	0.00	16.00	0.00	16.00	50.00	50.00
102R	0.00	16.00	0.00	0.00	16.00	50.00	50.00
103	0.00	0.00	20.00	0.00	20.00	50.00	50.00
103R	0.00	20.00	0.00	0.00	20.00	50.00	50.00
104	0.00	0.00	20.00	0.00	20.00	50.00	50.00
104R	0.00	20.00	0.00	0.00	20.00	50.00	50.00
105	0.00	0.00	28.00	0.00	20.00	50.00	50.00
105R	0.00	28.00	0.00	0.00	28.00	50.00	50.00
106	0.00	0.00	32.00	0.00	32.00	50.00	50.00
106R	0.00	32.00	0.00	0.00	32.00	50.00	50.00
107	0.00	0.00	36.00	0.00	36.00	50.00	50.00
107R	0.00	36.00	0.00	0.00	36.00	50.00	50.00
108A	0.00	0.00	6.80	0.00	20.00	25.37	74.63
108B	0.00	0.00	10.20	0.00	30.00	25.37	74.63
109A	0.00	0.00	6.80	0.00	20.00	25.37	74.63
109B	0.00	0.00	10.20	0.00	30.00	25.37	74.63
110A	0.00	0.00	6.80	0.00	20.00	25.37	74.63
110B	0.00	0.00	10.20	0.00	30.00	25.37	74.63

116	0.00	0.00	16.00	0.00	5.60	74.07	25.93
116R	0.00	16.00	0.00	0.00	5.60	74.07	25.93
117	0.00	0.00	20.00	0.00	7.00	74.07	25.93
117R	0.00	20.00	0.00	0.00	7.00	74.07	25.93
118	0.00	0.00	24.00	0.00	8.40	74.07	25.93
118R	0.00	24.00	0.00	0.00	8.40	74.07	25.93
119	0.00	0.00	28.00	0.00	9.80	74.07	25.93
119R	0.00	28.00	0.00	0.00	9.80	74.07	25.93
120	0.00	0.00	24.00	0.00	0.42	98.28	1.72
120R	0.00	24.00	0.00	0.00	0.42	98.28	1.72
121	0.00	0.00	28.00	0.00	0.49	98.28	1.72
121R	0.00	28.00	0.00	0.00	0.49	98.28	1.72
122	0.00	0.00	32.00	0.00	0.56	98.28	1.72
122R	0.00	32.00	0.00	0.00	0.56	98.28	1.72
127	0.00	0.00	24.00	0.00	1.20	95.24	4.76
127R	0.00	24.00	0.00	0.00	1.20	95.24	4.76
128	0.00	0.00	28.00	0.00	1.40	95.24	4.76
128R	0.00	28.00	0.00	0.00	1.40	95.24	4.76
129	0.00	0.00	32.00	0.00	1.60	95.24	4.76
129R	0.00	32.00	0.00	0.00	1.60	95.24	4.76
130	0.00	0.00	36.00	0.00	1.80	95.24	4.76
130R	0.00	36.00	0.00	0.00	1.80	95.24	4.76
131	0.00	0.00	24.00	0.00	1.68	93.46	6.54
131R	0.00	24.00	0.00	0.00	1.68	93.46	6.54
132	0.00	0.00	28.00	0.00	1.96	93.46	6.54
132R	0.00	28.00	0.00	0.00	1.96	93.46	6.54
133	0.00	0.00	32.00	0.00	2.24	93.46	6.54
133R	0.00	32.00	0.00	0.00	2.24	93.46	6.54
134	0.00	0.00	36.00	0.00	2.52	93.46	6.54
134R	0.00	36.00	0.00	0.00	2.52	93.46	6.54
135	0.00	0.00	76.80	0.00	1.68	97.86	2.14
135R	0.00	24.00	0.00	0.00	1.68	93.46	6.54
136	0.00	0.00	89.60	0.00	1.96	97.86	2.14
136R	0.00	28.00	0.00	0.00	1.96	93.46	6.54
137	0.00	0.00	102.40	0.00	2.24	97.86	2.14
137R	0.00	32.00	0.00	0.00	2.24	93.46	6.54
138	0.00	0.00	115.20	0.00	2.52	97.86	2.14
138R	0.00	36.00	0.00	0.00	2.52	93.46	6.54
139	0.00	0.00	30.00	0.00	1.50	95.24	4.76
139R	0.00	30.00	0.00	0.00	1.50	95.24	4.76
140	0.00	0.00	30.00	0.00	1.50	95.24	4.76
140R	0.00	30.00	0.00	0.00	1.50	95.24	4.76
141	0.00	0.00	10.00	0.00	0.50	95.24	4.76
141R	0.00	10.00	0.00	0.00	0.50	95.24	4.76
142	0.00	0.00	10.00	0.00	0.50	95.24	4.76
142R	0.00	10.00	0.00	0.00	0.50	95.24	4.76
143	0.00	0.00	24.00	0.00	1.20	95.24	4.76
143R	0.00	24.00	0.00	0.00	1.20	95.24	4.76
144	0.00	0.00	28.00	0.00	1.40	95.24	4.76
144R	0.00	28.00	0.00	0.00	1.40	95.24	4.76
145	0.00	0.00	32.00	0.00	1.60	95.24	4.76
145R	0.00	32.00	0.00	0.00	1.60	95.24	4.76
146	0.00	0.00	36.00	0.00	1.80	95.24	4.76
146R	0.00	36.00	0.00	0.00	1.80	95.24	4.76

147	0.00	0.00	28.00	0.00	1.40	95.24	4.76
147R	0.00	28.00	0.00	0.00	1.40	95.24	4.76
148	0.00	0.00	32.00	0.00	1.60	95.24	4.76
148R	0.00	32.00	0.00	0.00	1.60	95.24	4.76
149	0.00	0.00	7.68	0.00	0.36	95.52	4.48
149R	0.00	7.68	0.00	0.00	0.36	95.52	4.48
150	0.00	0.00	8.96	0.00	0.42	95.52	4.48
150R	0.00	8.96	0.00	0.00	0.42	95.52	4.48
151	0.00	0.00	10.24	0.00	0.48	95.52	4.48
151R	0.00	10.24	0.00	0.00	0.48	95.52	4.48
152	0.00	0.00	11.52	0.00	0.54	95.52	4.48
152R	0.00	11.52	0.00	0.00	0.54	95.52	4.48
153	0.00	0.00	0.00	0.00	0.00	0.00	0.00
154	0.00	0.00	76.80	0.00	0.00	100.00	0.00
154R	0.00	20.00*	0.00	0.00	0.00	100.00	0.00
155	0.00	0.00	89.60	0.00	0.00	100.00	0.00
155R	0.00	20.00	0.00	0.00	0.00	100.00	0.00
156	0.00	0.00	102.40	0.00	0.00	100.00	0.00
156R	0.00	20.00*	0.00	0.00	0.00	100.00	0.00
157	0.00	0.00	115.20	0.00	0.00	100.00	0.00
158	0.00	0.00	64.00	0.00	0.00	100.00	0.00
159	0.00	0.00	20.00	0.00	0.00	100.00	0.00

EXPLANATION OF CODES

R - REFERENCE
NO - NOT OSCILLATING
NA - NOT APPLICABLE
NR - OSCILLATING, BUT NO RESPONSE AFTER SAMPLE INTRODUCTION
OT - OVERTONE

TABLE 19

CRYSTAL COATING SOLUTIONS USED IN THE anti-DMMP STUDY

CRYSTAL #	CONCENTRATION OF PROTEIN				GLUT	TOTAL VOLUME PER SIDE	
	DEXTRAN mg/ml	BSA mg/mL	IgG mg/mL	aDMMP mg/mL	%	PROTEIN µL	GLUT µL
160	0.00	0.00	0.00	10.00	0.50	1.20	0.12
160R	10.00	0.00	0.00	0.00	0.50	1.20	0.12
161	0.00	0.00	0.00	10.00	0.50	1.40	0.14
161R	0.00	10.00	0.00	0.00	0.50	1.20	0.12
162	0.00	0.00	0.00	10.00	0.50	1.60	0.16
162R	0.00	0.00	10.00	0.00	0.50	1.20	0.12
163	0.00	0.00	0.00	10.00	0.50	1.80	0.18
164	10.00	0.00	0.00	0.00	0.50	1.20	0.12
164R	10.00	0.00	0.00	10.00	0.50	2.40	0.14
165	0.00	10.00	0.00	0.00	0.50	1.20	0.12
165R	0.00	0.00	0.00	10.00	0.50	1.20	0.12
166	10.00	10.00	0.00	10.00	0.50	3.60	0.24
166R	0.00	0.00	10.00	0.00	0.50	1.20	0.12
167	10.00	10.00	10.00	0.00	0.50	3.60	0.16
167R	10.00	10.00	10.00	10.00	0.50	4.80	0.36
168	0.00	10.00	0.00	10.00	0.50	2.40	0.24
168R	0.00	10.00	0.00	0.00	0.50	1.20	0.12
169	0.00	10.00	0.00	10.00	0.50	2.50	0.24
169R	0.00	10.00	0.00	0.00	0.50	1.50	0.12
170	0.00	10.00	0.00	10.00	0.50	2.00	0.20
170R	10.00	0.00	0.00	0.00	0.50	2.00	0.20
171	0.00	10.00	0.00	10.00	0.50	2.40	0.24
171R	10.00	0.00	0.00	0.00	0.50	2.40	0.24
172	0.00	10.00	0.00	10.00	0.50	2.80	0.28
172R	10.00	0.00	0.00	0.00	0.50	2.80	0.28
173	0.00	10.00	0.00	10.00	0.50	3.20	0.32
173R	10.00	0.00	0.00	0.00	0.50	3.20	0.32
174	0.00	0.00	0.00	10.00	0.50	1.60	0.16
174R	5.00	0.00	0.00	0.00	0.50	1.60	0.16
175	0.00	0.00	0.00	10.00	0.50	1.80	0.18
176	0.00	0.00	0.00	10.00	0.50	2.00	0.20
177	0.00	0.00	0.00	36.00	0.50	1.60	0.16
178	0.00	0.00	0.00	36.00	0.50	1.80	0.18

EXPLANATION OF CODES

R - REFERENCE
aDMMP - ANTI-DMMP ANTIBODY

TABLE 20

FINAL CRYSTAL COATINGS USED IN THE anti-DMMP STUDY

CRYSTAL	PROTEIN/CRYSTAL				GLUT	TOTAL WT %	
#	DEXTRAN mg	BSA mg	IgG mg	aDMMP mg	mg	PROTEIN %	GLUT %
160	0.00	0.00	0.00	24.00	1.20	95.24	4.76
160R	24.00	0.00	0.00	0.00	1.20	95.24	4.76
161	0.00	0.00	0.00	28.00	1.40	95.24	4.76
161R	0.00	24.00	0.00	0.00	1.20	95.24	4.76
162	0.00	0.00	0.00	32.00	1.60	95.24	4.76
162R	0.00	0.00	24.00	0.00	1.20	95.24	4.76
163	0.00	0.00	0.00	36.00	1.80	95.24	4.76
164	24.00	0.00	0.00	0.00	1.20	95.24	4.76
164R	24.00	0.00	0.00	24.00	1.40	97.17	2.83
165	0.00	24.00	0.00	0.00	1.20	95.24	4.76
165R	0.00	0.00	0.00	24.00	1.20	95.24	4.76
166	24.00	24.00	0.00	24.00	2.40	96.77	3.23
166R	0.00	0.00	24.00	0.00	1.20	95.24	4.76
167	24.00	24.00	24.00	0.00	1.60	96.77	3.23
167R	24.00	24.00	24.00	24.00	3.60	96.39	3.61
168	0.00	24.00	0.00	24.00	2.40	95.24	4.76
168R	0.00	24.00	0.00	0.00	1.20	95.45	4.55
169	0.00	20.00	0.00	30.00	2.40	95.42	4.58
169R	0.00	30.00	0.00	0.00	1.20	96.15	3.85
170	0.00	20.00	0.00	20.00	2.00	95.24	4.76
170R	40.00	0.00	0.00	0.00	2.00	95.24	4.76
171	0.00	24.00	0.00	24.00	2.40	95.24	4.76
171R	48.00	0.00	0.00	0.00	2.40	95.24	4.76
172	0.00	28.00	0.00	28.00	2.80	95.24	4.76
172R	56.00	0.00	0.00	0.00	2.80	95.24	4.76
173	0.00	32.00	0.00	32.00	3.20	95.24	4.76
173R	64.00	0.00	0.00	0.00	3.20	95.24	4.76
174	0.00	0.00	0.00	32.00	1.60	95.24	4.76
174R	16.00	0.00	0.00	0.00	1.60	90.90	9.10
175	0.00	0.00	0.00	36.00	1.80	95.24	4.76
176	0.00	0.00	0.00	40.00	2.00	95.24	4.76
177	0.00	0.00	0.00	115.20	1.60	98.63	1.37
178	0.00	0.00	0.00	129.60	1.80	98.63	1.37

EXPLANATION OF CODES

R - REFERENCE
NO - NOT OSCILLATING
NA - NOT APPLICABLE
NR - OSCILLATING, BUT NO RESPONSE AFTER SAMPLE INTRODUCTION
OT - OVERTONE

TABLE 21

RESPONSE WITH anti-DMMP COATED CRYSTALS

CRYSTAL	TOTAL COATING/CRYSTAL		RESPONSE[a]	Ra - Rr[b]
#	µg	Hz	Hz	Hz
90	12.24	23300	125.00	
90R	12.24	26209	15.13	109.87
91	2.00	9048	5.80	
91R	2.00	20T	NR	
92	0.20	10526	NR	
92R	0.20	16963	NR	
93	0.02	NO	NR	
93R	0.02	NO	NR	
94	0.002	NO	NR	
94R	0.002	NO	NR	
95	0.0002	4936	NR	
95R	0.0002	NO	NR	
96	40.00	21219	27 4	
96R	40.00	31179	30.4	- 3.00
97	48.00	26070	NR	
97R	48.00	37464	NR	
98	64.00	35776	33.75	
98R	64.00	49272	37.75	- 4.00
99	72.00	-26496	28.40	
99R	72.00	48829	34.00	- 5.60
100	16.00	12881	17.40	
100R	16.00	17907	21.40	- 4.00
101	24.00	18917	19.30	
101R	24.00	NO	NR	
102	32.00	19954	15.80	
102R	32.00	39352	27.40	- 11.60
103	40.00	20716	18.80	
103R	40.00	50246	33.40	- 14.60
104	40.00	30321	20.39	
104R	40.00	NO	NR	
105	56.00	38895	38.59	
105R	56.00	69456	51.40	- 12.81
106	64.00	44179	33.67	
106R	64.00	67323	58.10	- 24.43
107	72.00	68763	45.47	
107R	72.00	NO	NR	
108A	26.80	26319	41.50	
108B	40.20	42585	28.32	
109A	26.80	53060	NR	
109B	40.20	80119	NR	
110A	26.80	NO	NR	
110B	40.20	NO	NR	
116R	21.60	21021	55.70	- 43.60
117	27.00	41108	18.56	

117R	27.00	33040	74.37	- 55.81
118	32.40	24168	11.75	
118R	32.40	44245	105.21	- 93.46
119	37.80	4040	9.75	
119R	37.80	24462	70.41	- 60.66
120	24.42	41659	46.70	
120R	24.42	18703	12.67	34.03
121	28.49	51005	58.80	
121R	28.49	24423	20.57	38.23
122	32.56	28300	51.69	
122R	32.56	53432	18.47	33.22
127	25.20	40783	77.53	
127R	25.20	12291	20.76	56.77
128	29.40	52058	75.51	
128R	29.40	17193	13.09	62.42
129	33.60	56409	79.17	
129R	33.60	19397	13.79	65.38
130	37.80	- 6530	78.88	
130R	37.80	20381	20.93	57.95
131	25.68	40706	75.18	
131R	25.68	29489	45.29	29.89
132	29.96	53957	104.20	
132R	29.96	20305	30.32	73.88
133	34.24	62020	120.98	
133R	34.24	28726	37.56	83.42
134	38.52	62374	126.65	
134R	38.52	37875	46.00	80.65
135	78.48	36740	69.33	
135R	25.68	52677	96.00	- 26.67
136	91.56	48156	85.00	
136R	29.96	14852	27.18	57.82
137	104.64	52758	87.20	
137R	34.24	3913	81.60	5.60
138	117.72	53674	81.84	
138R	38.32	20512	24.12	57.72
139	96.15	48995	58.25	
139R	31.50	21870	16.50	41.75
140	96.15	55115	27.00	
140R	31.50	21870	6.75	20.25
141	10.50	16817	92.00	
141R	10.50	14483	61.60	30.40
142	10.50	11400	51.25	
142R	10.50	-187323	57.75	- 6.50
143	25.20	13990	59.00	
143R	25.20	19861	80.20	- 21.20
144	29.40	14031	51.60	
144R	29.40	24757	103.60	- 52.00
145	33.60	15058	58.20	
145R	33.60	34852	195.00	-136.80
146	37.80	17360	54.25	
146R	37.80	51771	287.50	-233.25
147	29.40	15236	66.60	
147R	29.40	19348	56.60	10.00
148	33.60	16772	51.20	

148R	33.60	32175	102.60	- 51.40
149	8.04	17666	69.60	
149R	8.04	11584	43.60	26.00
150	9.38	24995	82.40	
150R	9.38	13588	33.20	49.20
151	10.72	32274	71.00	
151R	10.72	15103	28.00	49.20
152	12.06	31506	77.40	
152R	12.06	11461	40.00	37.40
153	0.00	0	15.00	
154	76.80	54882	275.80	
154R	20.00	16106	92.81	182.99
155	89.60	66313	316.36	
155R	20.00	10216	59.76	246.60
156	102.40	70860	341.80	
157	115.20	- 2275	231.41	
158	64.00	71306	157.35	
159	20.00	14786	49.77	
160	25.20	17021	10.80	
160R	25.20	18499	12.80	2.00
161	29.40	23457	6.80	
161R	25.20	19000	6.40	0.40
162	33.60	29173	2.00	
162R	25.20	33826	2.00	0.00
163	37.80	28463	4.50	
164	25.20	22005	27.40	
164R	49.40	24205	30.40	- 3.00
165	25.20	34714	35.90	
165R	25.20	17492	19.50	16.40
166	74.40	41537	51.63	
166R	25.20	38933	37.40	14.23
167	74.40	0	NO	
167R	99.00	13423	62.90	
168	50.40	58988	NT	
168R	26.40	0	NO	
169	52.40	44186	NT	
169R	31.20	49362	NT	
170	42.00	23098	54.85	
170R	42.00	31300	26.77	28.08
171	50.40	20329	47.80	
171R	50.40	31793	35.44	12.36
172	58.80	36811	56.18	
172R	58.80	32914	32.47	23.71
173	67.20	29666	68.63	
173R	67.20	66599	NO	
174	33.60	24097	26.80	
174R	17.60	26009	14.63	12.17
175	37.80	29230	28.33	
176	42.00	27437	24.75	
177	116.80	59543	100.00	
178	131.40	85970	124.50	

EXPLANATION OF CODES

R - REFERENCE
NO - NOT OSCILLATING
NA - NOT APPLICABLE
NR - OSCILLATING, BUT NO RESPONSE AFTER SAMPLE INTRODUCTION
OT - OVERTONE
Ra - ACTIVE RESPONSE
Rr - REFERENCE RESPONSE

[a] Response to DMMP 0.9 ppm
Flow rate - 100 mL/min

[b] Active response - reference response

TABLE 22

EVALUATION OF SCRUBBERS TO REMOVE MOISTURE

SCRUBBER	RESPONSE (Hz)[a]	RESPONSE EFFICIENCY[b]
Na_2SO_4	212	89%
$ZnSO_4$	62	26%
Na_2SO_3	98	42%

[a] Response to 0.9 ppm DMMP

[b] At 50% relative humidity