complexation of Cd(II) by several classes of hydrazides including isonicotinic hydrazide (*5*) occurred through a totally different route. No protons were released in the overall reaction; at least three or four soluble complexes were observed; and a symmetrically disubstituted hydrazide, diacetyl hydrazine, showed no evidence of complexation. These data indicate that complexation must occur through a neutral hydrazide moiety and cannot involve chelation; the cadmium complex must be of a monodentate form. This is unusual since the zinc complex was reported to be a chelate (*3*) and one would expect, in general, similar behavior from both cadmium and zinc. Accordingly, a critical reevaluation of metal-hydrazide complexes was undertaken, particularly the Cu(II)-hydrazide systems.

Titration of solutions of various salts of Cu(II) with solutions of various hydrazides, including isonicotinic hydrazide, does produce a significant drop in *p*H below that observed for either metal ion or hydrazide alone. In addition, precipitation during titration was observed with isonicotinic hydrazide and nicotinic hydrazide, and effervescence was noted during the Cu(II)–isonicotinic hydrazide titration. Examination by gas chromatography indicated that the evolved gas was nitrogen. From a solution containing 2 mmole of isonicotinic hydrazide and 1 mmole of $CuCl_2$, 0.3 mmole of N_2 gas were collected, although no attempt was made to capture all the nitrogen. The quantity of evolved nitrogen thus indicates that a major reaction occurred.

Another solution containing 10 mmole of isonicotinic hydrazide and 5 mmole of $CuCl_2$ was prepared, and the brown precipitate which was produced was filtered, washed, and dried. The solid had a melting point of 265°C and was insoluble in water, alcohol, acetone, carbon tetrachloride, and benzene, but it could be dissolved in 5 percent hydrochloric acid. Infrared spectra of the precipitate indicated the presence of both carbonyl and N-H groups. These data and elemental analyses are consistent with a material containing equimolar quantities of copper, isonicotinic hydrazide, and chlorine, that is, Cu • RH • Cl. The presence of one chloride demonstrates that the copper–isonicotinic hydrazide fragment is a positively charged (+1) species.

Thus it appears that an oxidation-reduction reaction occurs between $CuCl_2$ and isonicotinic hydrazide to produce nitrogen and a reduced form of copper. The reaction of hydrazides with metal ions [such as Cu(II)] to produce nitrogen is well documented and has been used for the analysis of hydrazides for many years (*6*, *7*).

As a further step toward elucidating the overall reaction, we may postulate that the reduced form of copper can react with another mole of hydrazide to give the very insoluble brown precipitate. Samples of the precipitate were treated with *p*-dimethylaminobenzalrhodanine (*8*) and 2,2′-biquinoline (*9*). In both cases, a strong positive test for Cu(I) was obtained. Electron paramagnetic resonance spectra, however, disclosed the presence of some Cu(II) in the precipitate. An iodometric determination of the Cu(II) content disclosed the presence of 2.1 percent Cu(II) (by weight) out of a total copper content of 25.2 percent. Therefore, approximately 92 percent of the copper which was precipitated as the copper–isonicotinic hydrazide complex existed in the Cu(I) form. Whether the 8 percent Cu(II) is due to occluded material, a reoxidation of the cuprous ion, or an equilibrium between Cu(I) and Cu(II) species is not known. Other analyses showed, however, that exposure of the precipitate to an oxygen atmosphere increased the Cu(II) assay. Furthermore, a precipitate with essentially the same characteristics as those described above was produced by reaction of CuCl and isonicotinic hydrazide.

These findings, therefore, point toward an initial reaction of Cu(II) with isonicotinic hydrazide to give Cu(I), followed by the formation of an insoluble Cu(I)–isonicotinic hydrazide–chloride complex.

The presence of copper enhances the antitubercular activity of isonicotinic hydrazide. This enhancement was postulated to be due to a neutral 2 : 1 Cu(II) complex which might penetrate the bacterial cell wall more readily than the hydrazide alone. However, the results of this investigation indicate that the copper is reduced to Cu(I) ion, and that a charged 1:1 hydrazide complex is then formed. Therefore, it seems more reasonable to ascribe the synergistic effect of copper to the initial oxidation-reduction reaction that yields Cu(I), or alternatively, to the greater toxicity of the cuprous hydrazide complex against *Mycobacterium tuberculosis*. The same mechanism might account for a similar synergistic effect of copper when used with another antitubercular drug, *p*-acetamidobenzaldehyde thiosemicarbazone (thiacetazone) (*10*).

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References and Notes

1. E. Sorkin, W. Roth, H. Erlenmeyer, *Helv. Chim. Acta* **35**, 1736 (1952).
2. S. Fallab and H. Erlenmeyer, *Experientia* **8**, 298 (1952); *Helv. Chim. Acta* **36**, 6 (1953).
3. A. Albert, *Experientia* **9**, 370 (1953).
4. ———, *Nature* **177**, 525 (1956).
5. A. F. Krivis, G. R. Supp, R. L. Doerr, *Anal. Chem.* **37**, 52 (1965); *ibid.* **38**, 936 (1966); G. R. Supp, *ibid.* **40**, 981 (1968).
6. H. Strache, *Monatsh. Chem.* **12**, 524 (1891); H. Harting, *J. Amer. Pharm. Ass.* **42**, 323 (1953).
7. S. Siggia, *Quantitative Organic Analysis via Functional Groups* (Wiley, New York, ed. 3, 1965).
8. F. Feigl, *Spot Tests in Inorganic Analysis* (Elsevier, Amsterdam, ed. 5, 1958).
9. I. M. Kolthoff and P. J. Elving, Eds., *Treatise on Analytical Chemistry* (Interscience, New York, 1961), part 2, vol. 3.
10. K. Liebermeister, *Z. Naturforsch.* **5b**, 79 (1950).

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Maser Amplification of 9.5-Gigahertz Elastic Waves in Sapphire Doped with Divalent Nickel Impurity Ions

Abstract. *The three spin energy levels of divalent nickel impurity ions in sapphire interact strongly with ultrasonic waves whose frequency corresponds to certain allowed transitions between the levels. Under population inversion of the levels it is possible to achieve significant amplification of very high frequency ultrasonic waves by stimulated emission from the spin system.*

This report describes a successful attempt to achieve the amplification of 9.5-Ghz longitudinal elastic waves in sapphire by stimulated emission from the inverted spin population of Ni^{2+} impurity ions. Phonon maser action by impurity spin systems has been reported by Tucker (*1*), who worked with ruby, and by Shiren (*2*), who employed adiabatic fast passage to invert the spin population of Fe^{2+} in MgO.

The choice of Ni^{2+} as a phonon

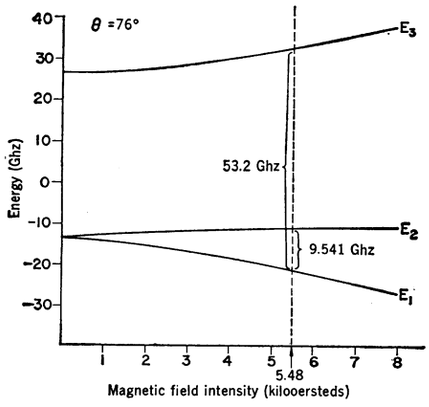

Fig. 1. Energy-level diagram of Ni^{2+} in sapphire versus magnetic field intensity. The angle between the field direction and the C-axis is 76°. The operating conditions for maser action are indicated.

amplifier seemed particularly suitable because one expects in this case a spin-lattice coupling of intermediate strength. On the basis of measurements on Ni^{2+} in MgO (*3*), it could be inferred that the spin-phonon coupling of Ni^{2+} in sapphire would be strong enough to achieve considerable gain, yet not so strong as to require excessive pump power for population inversion.

One disadvantage not appreciated by us at the conception of this project is the apparent difficulty, if not the impossibility, of growing sapphire doped with a Ni^{2+} concentration exceeding approximately 10 parts per million by either the flame fusion or Czochralski technique. In support of this, we failed to find in any of the several crystals supplied to us Ni^{2+} concentrations exceeding this amount, although we had requested concentrations of at least ten times this figure.

The spin-phonon interaction Hamiltonian has been described by Dobrov (*4*) in terms of the spin operators and the magnetoelasitc coupling constants. The salient feature of this equation is that the interaction between an elastic wave and the spin system depends importantly on the wave polarization and propagation direction, and on the angle of the magnetic field relative to crystal axes. From the above it is straightforward to derive the absorption coefficient, α, which describes the absorption or emission of sound quanta by the spin system when the phonon frequency matches the energy separation between a pair of levels m and m'. The sound wave intensity I versus distance of propagation x is then given by $I = I_0 e^{-\alpha x}$, which predicts attenuation for $\Delta n > 0$ (normal population) and amplification for $\Delta n < 0$ (inverted population). Here Δn is the excess spin population in the lower energy level per unit volume of the crystal.

Marshall *et al.* (*5*) have observed the microwave paramagnetic resonance absorption spectrum of divalent nickel in sapphire and have shown that the spectrum can be described by an appropriate spin Hamiltonian with effective spin $S = 1$. Using this Hamiltonian, we machine-calculated the eigenstates and energies of the spin system as functions of the magnetic field intensity, and the angle between the field direction and the C-axis. Figure 1 shows the result of these calculations for the three energy levels of Ni^{2+} versus magnetic field at an angle of 76° between field direction and C-axis. Moreover, these computations in concert with the terms in the spin-phonon interaction energy (*4*) indicated that for the transition (1,2) α would be several hundred times larger for longitudinal wave propagation along the A-axis as compared to propagation along the C-axis. [Both are pure mode axes for longitudinal waves (*6*).] Subsequent experiments supported this conclusion, showing strong ultrasonic absorption for propagation along the A-axis, and undetectable absorption along the C-axis. Hence we were led to attempt amplification of longitudinal waves propagating along the A-axis.

Our experimental arrangement was the following: A sapphire crystal doped with Ni^{2+} was fabricated in the shape of a right circular cylinder 16.9 mm long and 3.5 mm in diameter, with the A-axis perpendicular to the end faces (*7*). After the usual optical polishing of the end faces, one end received an evaporated CdS transducer film. The coated end was inserted in a reentrant cavity tuned to the (1,2) transition, whereas the bulk of the crystal filled a pump cavity (*8*) tuned to the (1,3) transition. (For our operating field of 5.48 kilooersteds the corresponding frequencies were 9.54 and 53.2 Ghz; see Fig. 1.) The crystal and cavities were held at about 2°K in a helium bath.

We employed the pulse-echo technique (*9*) to observe the fate of longitudinal elastic waves generated at 9.54 Ghz by the CdS film transducer. In order to avoid saturation of transition (1,2) the radio-frequency pulse power input to the reentrant cavity was limited to about ½ watt. At this input power level and with zero magnetic field and quiescent pump, only the first 12 echoes were visible. In contrast, Fig. 2 shows some 330 echoes as a result of gain. In this case the ultrasonic drive power remains the same, but the pump and magnetic field are set to the values indicated in Fig. 1. A pump power of some 150 mw was found entirely adequate. Ultrasonic pulses of 1-μsec width were delivered to the rod at a low repetition rate, typically 50 pulse/sec. The scale in Fig. 2 is 50 μsec per division, and the echoes are separated by 2.94 μsec. By comparing the 12th echo (with and without maser action) the gain is estimated to be 0.13 db/cm.

The first half of the echo train in Fig. 2 clearly indicates that the gain exceeds all losses. It would seem reasonable, therefore, to expect self-sustaining ultrasonic oscillations at the (1,2) transition. However, no such continuous wave output was detected.

The eventual decay of the echo train, even under conditions of net gain, appears to result from small crystalline imperfections and nonparallel ends which cause the ultrasonic wave to walk off the axis of the rod. These same defects may also explain, at least in part, the beating effect to be noted in the echo profile. However, we have also observed that the condition of the spin system drastically influences the beat pattern. This

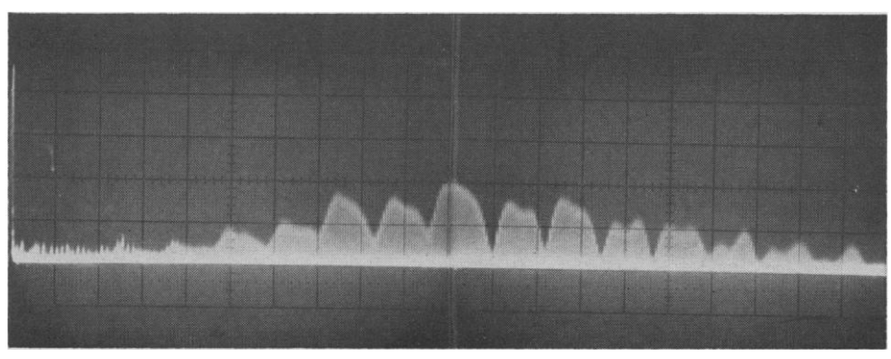

Fig. 2. Amplified echo train under the conditions indicated in Fig. 1. Sweep equals 50 μsec/cm.

interaction may be simply the result of dispersion, although we are not entirely satisfied with this explanation.

We believe that much higher Ni^{2+} concentrations, and thus higher gains, are possible with modified crystal-growing methods. Such a development would permit easy determination of all the magnetoelastic coupling constants and could greatly facilitate ultrasonic research at higher frequencies and with lossy materials.

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References and Notes

1. E. B. Tucker, *Phys. Rev. Letters* **6**, 547 (1961).
2. N. S. Shiren, *Appl. Phys. Letters (U.S.A.)* **7**, 142 (1965).
3. ———, in *Magnetic and Electric Resonance and Relaxation*, J. Smidt, Ed. (North-Holland, Amsterdam, 1963), pp. 114–122.
4. W. I. Dobrov, *Phys. Rev.* **134**, A734 (1964).
5. S. A. Marshall, T. T. Kikuchi, A. R. Reinberg, *ibid.* **125**, 453 (1962).
6. G. W. Farnell, *Can. J. Phys.* **39**, 65 (1961).
7. Prior to optical polishing of the end faces, the rod was exposed to a hydrogen atmosphere at 1200°C for several hours. This procedure reduced Ni^{3+} to Ni^{2+}, approximately doubling the concentration of the latter. The final Ni^{2+} content as determined by calibrated electron paramagnetic resonance measurements was approximately 5 parts per million.
8. Although the pump cavity was designed to operate in one of the $TE_{0,2,n}$ modes, there were in fact several other closely spaced modes in the neighborhood of 53 Ghz, which is a result of the large ratio of length to diameter of the cavity. Consequently, it was impossible to identify the particular mode being excited, so that in practice a cavity mode was chosen solely on the basis of strong coupling to the V-band pump.
9. The pulse-echo technique is described by E. B. Tucker, in *Physical Acoustics*, W. P. Mason, Ed. (Academic Press, New York, 1966), vol. 4, pt. A, pp. 77–78.
10. Work supported by NSF grant GP 6448. We thank Mr. Charles Sahagian of Air Force Cambridge Research Laboratories for providing the sapphire boule, Dr. John de Klerk of the Westinghouse Research Laboratories for graciously evaporating the CdS transducing films, and Dr. Theodore Castner of the University of Rochester for his active interest and many helpful discussions.

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Nucleoside Triphosphate Termini from RNA Synthesized in vivo by Escherichia coli

Abstract. *Alkaline hydrolyzates of RNA made in vivo by* Escherichia coli *contain ribonucleoside-3′-monophosphate-5′-triphosphates. These probably arise by hydrolysis of the initial nucleoside triphosphate from the 5′ terminus of the nascent RNA chains. Logarithmically growing cultures, labeled for 45 seconds with ^{32}P-labeled phosphate, yield about 2000 molecules of labeled tetraphosphate per cell, this yield increasing only slightly with continued labeling. Only the tetraphosphates of adenosine and guanosine have been found in* Escherichia coli, *and these two are present in approximately equal amounts.*

Studies in vitro of the DNA-primed RNA polymerase reaction have shown that RNA synthesis proceeds in the 5′ to the 3′ direction and that a triphosphate moiety is retained at the 5′ end of product RNA molecules (*1, 2*). In these cell-free experiments, RNA was synthesized with RNA polymerase and γ-labeled ^{32}P-nucleoside triphosphates (*pppX) (*3*) in the presence of a variety of DNA primers. Alkaline hydrolysis of the resulting RNA produced unlabeled nucleoside monophosphates (Xp) from the internal nucleotides of the molecules, a nucleoside (X) from the 3′-hydroxyl ends, and a ^{32}P-labeled nucleoside tetraphosphate from the 5′-triphosphate ends of the molecules (*pppXp). These studies have been performed with RNA polymerase from *Escherichia coli* and *Azotobacter vinlandii*. In both these systems, the RNA made was preferentially initiated by a purine ribonucleoside triphosphate (adenine or guanine), while the ratio of adenine to guanine termini was dependent on the source and physical state of the DNA used as primer.

Experiments in vivo have shown that the synthesis of RNA in *E. coli* is in the 5′ to 3′ direction, as in the in vitro systems (*4*). However, in *E. coli*, purine nucleotide monophosphates have been found at the 5′ termini of 23*S* ribosomal RNA (pGpX. . .), 16*S* ribosomal RNA (pApX. . .), and transfer RNA (pGpX. . .) (*5*). Terminal triphosphates have been found on the RNA of some RNA containing bacteriophage (*6*).

We have analyzed hydrolyzates of RNA from growing cultures of *E. coli* for the presence of pppXp terminal groups. Because of the length of RNA polymers and the probable short life of the terminal groups (they do not appear on the stable RNA species), we sought conditions to label the terminal phosphates selectively. With this in mind we administered ^{32}P-phosphate to cultures for short periods only. This procedure is based on the observation by Bolton and Roberts (*7*) that, after addition of ^{32}P-phosphate to a culture of *E. coli*, ^{32}P appears first in the β- and γ- phosphates of ATP and only after a lag in the α position. The *E. coli* strain ML 30 was grown at 37°C (*8*) and ^{32}P-phosphate was administered for periods between 45 and 120 seconds. The cells were then collected in 5 percent TCA, washed, and hydrolyzed with KOH. The protein, DNA, and K^+ were subsequently removed by addition of perchloric acid, and the remaining RNA nucleotides were applied to a DEAE-cellulose column and eluted with buffers containing 7*M* urea (*9*). Adenosine-5′-tetraphosphate (ppppA), which contained some ADP and ATP as well, or a ribonuclease digest of yeast RNA, was added to the samples to provide ultraviolet-absorbing markers for the chromatography. This system (*9*) has been useful for separating small oligonucleotides, but it is also convenient for separating mono-, di-, tri-, and tetraphosphomononucleotides (Fig. 1). The pyrimidines are eluted slightly ahead of the purines, the order of elution being cytidine, uridine, adenosine, guanosine, so that a double peak is observed when all four nucleotides are present (Figs. 1 and 2). Separate chromatography of the four nucleoside triphosphates in pairs showed that they are eluted in this order as well (Fig. 2a). In this experiment a pancreatic ribonuclease digest of RNA was added as a marker to facilitate comparison of these data to those of others who studied the elution of pppXp compounds in similar chromatographic systems (*5*).

In the RNA hydrolyzate from cells labeled for 2 minutes, a large amount of ^{32}P-label is present in the monophosphate region and in the di- and triphosphate regions as well (*10*), but there is little label in the eluent containing the ultraviolet-absorbing peak of ppppA. There is a distinct double peak of phosphate radioactivity in the region following the ppppA peaks where pppXp compounds with a net negative charge of 6 (*p*H 7.7) would be expected to be eluted (see below). The first half of this peak is referred to as ppp*I*p, and the latter half as ppp*II*p. In individual experiments there was a slight predominance of one or the other but in these regions from six different chromatograms, an average of 49 percent of the total radioactivity was in the ppp*I*p