A novel ultrasonic flow injection chemiluminescence (FI-CL) manifold for determining hydrogen peroxide ($H_2O_2$) has been designed\cite{1}. Chemiluminescence obtained from the luminol-$H_2O_2$-cobalt (II) reaction was enhanced by applying 120 W of ultrasound for a period of 4 s to the reaction coil in the FI-CL system and this enhancement was verified by comparison with an identical manifold without ultrasound. The method was applied to the determination of trace amounts of $H_2O_2$ in purified water and natural water samples without any special pre-treatments.

It is well-known that alkaline solutions of luminol emit light when subject to ultrasound of sufficient intensity to produce acoustic cavitation. Light emission is believed to occur through a process of oxidative chemiluminescence involving sonochemically generated $HO^\cdot$. The cyclic pressure variations associated with the propagation of ultrasound waves in aqueous solution are known to result in the growth and periodic collapse of microscopic cavitation bubbles filled with gas and/or vapour\cite{2}\cite{40}. Furthermore, it has been shown that extremely high local temperatures and pressures may be generated during the collapse or implosion of such bubbles. Consequently, it is generally accepted that it is within the cavitation bubble, or the layer of solution immediately contacting the cavitation bubble, that the sonochemical effects take place.

Luminol chemiluminescence has been described in section B1 (ADD LINK). Light emission from the reaction between luminol and hydrogen peroxide can be induced by the presence of cobalt(II) at concentrations low enough to be regarded as catalytic. The effect of ultrasound on hydrogen peroxide is to produce hydroxyl radicals by homolytic fission of the $O-O$ bond:

$$[H_2O_2 \rightarrow 2HO^\cdot]$$

Hydroxyl radicals in aqueous solution are short-lived. The consumption of these radicals by recombination is very rapid and attenuates the ultrasound enhancement:

$$[2HO^\cdot \rightarrow H_2O_2]$$

Because of this, the concentration of hydrogen peroxide soon greatly exceeds that of hydroxyl radical, even if the radicals are initially produced in high yield. There is then a greater probability that radicals will instead react with hydrogen peroxide molecules, forming superoxide:

$$[HO^\cdot + H_2O_2 \rightarrow O_2^{\cdot-} + H_3O^+]$$

As a result, the effect of sonication is the production in the sample of superoxide rather than hydroxyl radicals. The hydroxyl radicals initially formed would have reacted with luminol to initiate the light-emitting pathway but the primary oxidation of luminol by superoxide is negligible. Instead, when the sample merges with luminol/buffer/cobalt, the effect of this enhanced superoxide concentration is to increase the concentration of the hydroperoxide intermediate, enhancing the light emitting pathway where it has already been initiated by cobalt/hydrogen peroxide; this leads to a fivefold improvement in the detection limit.

The practical implementation of this ultrasound enhancement proved to be exacting. Small changes in the FIA manifold were found to have a considerable effect on the chemiluminescence intensity. In spite of this it was found possible to optimize a range of relevant variables. Some variables were concerned with the arrangements for administering a dose of ultrasound energy to the sample as it flowed through a coil immersed in the sonication bath. To achieve this, the coil had
to be long enough to contain the sample all the time that sonication was occurring, but not so long that the enhancement of the chemiluminescence signal would be abolished either by dispersion of the sample into the carrier or by decay of the short-lived radicals generated by sonication. The optimum distances between the water surface and the probe tip and between the probe tip and the upper edge of the sonication coil correspond closely to the conditions for the establishment of standing waves in the sonic bath.

Cavitation when present is the predominant mechanism of acoustic energy absorption as well as providing the collapsing bubbles that are the sites of the sonochemical reactions. Absorption by bubbles is so effective that they provide a shielding effect and so could explain the difficulty in predicting the effect of small changes in the position of the coil within the sonic bath. It was necessary to vary the sonication arrangements in order to optimise them, but operational analytical applications of ultrasound enhancement would be more easily carried out using fixed sonication arrangements in a permanent and purpose-designed apparatus.