Feature Article

Biosensors Based on Aligned Carbon Nanotubes Coated with Inherently Conducting Polymers

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Abstract

The use of multiwalled aligned carbon nanotubes provides a novel electrode platform for inherently conducting polymer based biosensors. The example used here to highlight the usefulness of such a platform is the polypyrrole based glucose oxidase system for detection of glucose. The use of these three dimensional electrodes offers advantages in that large accessible enzyme loadings can be obtained within an ultrathin layer. It has also been found that the detection of H_2O_2 at these new electrode structures containing iron loaded nanotube tips can be achieved at low anodic potentials. The result is a sensitive and selective glucose sensor.

Keywords: Aligned carbon nanotubes, Glucose oxidase, Hydrogen peroxide

1. Introduction

Since the discovery of carbon nanotubes [1], there has been intensive research aimed at exploiting their mechanical and electrical properties [2]. Applications of interest include their use in supercapacitors [3], field emission displays [4], hydrogen storage systems [5], artificial muscles [6] and as novel platforms for cell culturing [7]. Many of these applications depend on the electrochemical properties of carbon nanotubes. Individual carbon nanotubes themselves have been reported to be highly conductive with conductivities up to 5000 S cm^{-1} while mats composed of entangled nanotubes have conductivities of the order of 200–300 S cm^{-1} [6]. It has also been shown that the electrochemistry is predominately capacitive in nature (values in excess of 50 F/g have been reported), however responses due to the presence of surface bound redox responses (quinone-hydroquinone) have also been observed [8–10]. More recently aligned carbon nanotube structures have been produced [11, 12]. It has been shown that these materials display electrochemical properties similar to single walled nanotube (SWNT) mats in that they are highly capacitive in nature [13].

Studies into the electrochemistry of redox couples using carbon nanotube (CNT) electrodes are limited, however, one report does show the oxidation/reduction of dopamine to be more facile compared with other carbon surfaces [14] and another [15] shows that using CNT electrodes allows direct access to the redox center in cytochrome c. These useful electrochemical properties combined with the high surface area available with CNT based electrodes should provide some advantages in a number of electrochemical sensing areas. In addition, it has been shown that the chemical environment to which nanotubes are exposed has an effect on the inherent electronic properties of carbon nanotubes [16] providing an alternative or additional transduction mechanism. Such properties suggest that the use of CNTs as platforms to build new sensing technologies is an area worthy of investigation.

The electrochemical properties of aligned CNT arrays have been utilized in the formation of conducting polymer-carbon nanotube (CP-CNT) coaxial nanowire composites. This array of polymer coated nanotubes is produced by electrodeposition of a concentric layer of conducting polymer onto individual aligned nanotubes resulting in a structure with large electroactive surface area [17].

The development of biosensors based on inherently conducting polymers (ICPs) remains an area of great interest. ICPs such as polypyrroles (I, Scheme 1) provide an excellent platform upon which to build biorecognition and/or signal transduction for the target analyte.

Biomolecular components such as enzymes (18, 19), antibodies [20], oligonucleotides [21] and even whole living cells [22] have been incorporated into the polymer (as the dopant (A$^-$)) at the time of synthesis. The intimate contact of the biocomponent with the polymer enables efficient signal transduction. In the case of electrochemical immunosensors [20], using antibodies as the biomolecular recog-
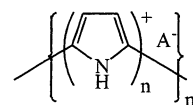
(I ≡ Polypyrrole, n = 3-4, m determines molecular weight)

Scheme 1.

nition element, the analytical signal arises from changes in capacitance as the target antigen interacts with the bioactive polymer. In the case of enzyme-containing inherently conducting polymers, the detection of H_2O_2 or some other electroactive product generated by the enzymatic reaction is the basis of signal generation [18, 19]. To maximize sensitivity and selectivity respectively it is important that sufficient quantities of H_2O_2 are generated per unit time and that the product is detected at as low a potential as possible. For previous polypyrrole-based sensors containing glucose oxidase, it has been noted that the potential required to oxidize H_2O_2 results in overoxidation of polypyrrole and also is sufficient to oxidize common interferents such as ascorbic acid and urea [23].

In the course of this work we have investigated the use of aligned carbon nanotubes as a platform for production of a conducting polymer-glucose oxidase based biosensor. This has been achieved by formation of a bioactive conducting polymer coaxial sheath around individual aligned carbon nanotubes. The resultant structures have been shown to have good electrochemical properties with the bioactivity of the enzyme incorporated into the conducting polymer during growth being retained.

2. Experimental

Glucose oxidase (GOX, 15500 units g^{-1}) was purchased from Sigma. Pyrrole (Aldrich) was distilled prior to use. A 0.10 M buffer solution (pH 7.45) was prepared from sodium phosphate in MilliQ water. All other chemicals were commercially obtained and used without further purification.

The aligned carbon nanotube films were prepared by pyrolyzing iron (II) phthalocyanine under Ar/H_2 at 900 °C as described in detail elsewhere [11]. To construct a gold-supported nanotube microelectrode array for electrochemical synthesis of the CP-CNT coaxial nanowires, a thin layer of gold was sputter coated onto the amorphous carbon layer that covers an as-synthesized aligned nanotube array grown on a quartz glass plate. The gold layer supported nanotube array was then separated from the quartz substrate with an aqueous solution of HF (30% w/w).

SEM images were obtained using a Philips XL-30 FEG SEM unit at 5 KV. XPS analyses were performed on a Kratos Analytical spectrometer using monochromatic $Al_{K\alpha}$ radiation at a power of 200 W. All electrochemical studies were carried out using a MacLab/4E analyzer. A single-compartment cell with a working volume of 15 mL was used. A strip of aligned carbon nanotube array (0.09 cm²) was used as a working electrode, and Ag/AgCl and platinum foil used as reference and counter electrodes respectively.

GOX immobilization was performed by oxidation of pyrrole (0.10 M) in a solution containing 0.10 M $NaClO_4$, and 2 mg/mL GOX, in pH 7.45 buffer solution at 10 °C. A constant potential of 1 V was employed for the electropolymerization (1 min). The amperometric response of the prepared electrodes to glucose was examined in 0.10 M

phosphate buffer solution (pH 7.45) by measuring the oxidation current at a potential of 0.45 V. The background current was allowed to stabilize for at least 30 min before injection of glucose to a stirred solution. The calibration of the sensor was obtained by adding increasing amounts of glucose and the steady state current was recorded.

3. Results and Discussion

Scanning electron micrographs of aligned carbon nanotube arrays were obtained using the method described in Section 2. Nanotube lengths as long as 20 μm and with diameters of approximately 50 nm were obtained. Electrochemical studies using $Fe(CN)_6^{4-}$ as a redox probe indicated that a reversible oxidation-reduction process (ratio of oxidation/reduction current = 1) was observed under cyclic voltammetric conditions with a scan rate of 50 mV/sec. A higher than expected peak-to-peak separation of 0.11 V was observed using the aligned CNT array and this may be attributed to the fact that iR drop effects are exacerbated compared to a conventional metal electrode with lower conductivity expected and higher current densities observed. The much higher current density (id) observed with the CNT electrode ($id = 1.07$ mA/cm²) compared to the glassy carbon electrode ($id = 0.08$ mA/cm²) when geometric surface areas are used to calculate these values indicates the greater electroactive area available along the length of the tubes.

Since the electrochemical detection of glucose using the glucose oxidase-containing electrode involves oxidation of H_2O_2 [22], this electrochemical process was also investigated at aligned carbon nanotube electrodes. An irreversible single oxidation peak was observed at approximately 0.30 V
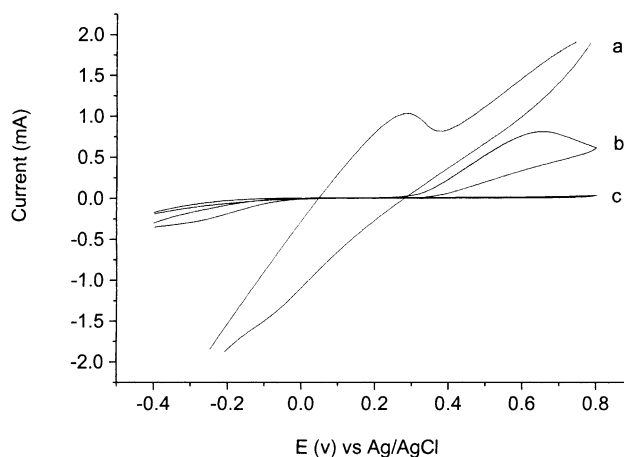

Fig. 1. Cyclic voltammograms obtained for 0.10 M H_2O_2 buffer solution (pH 7.45) at a) an aligned CNT array electrode containing Fe nanoparticle cap; b) a gold film electrode prepared by sputter coating on a plastic substrate; c) an aligned CNT electrode in buffer only without H_2O_2 present. Each electrode was scanned for 2 cycles in buffer with gentle stirring before injection of H_2O_2. The geometric surface area for each electrode is 0.09 cm². Scan rate = 50 mV/s.
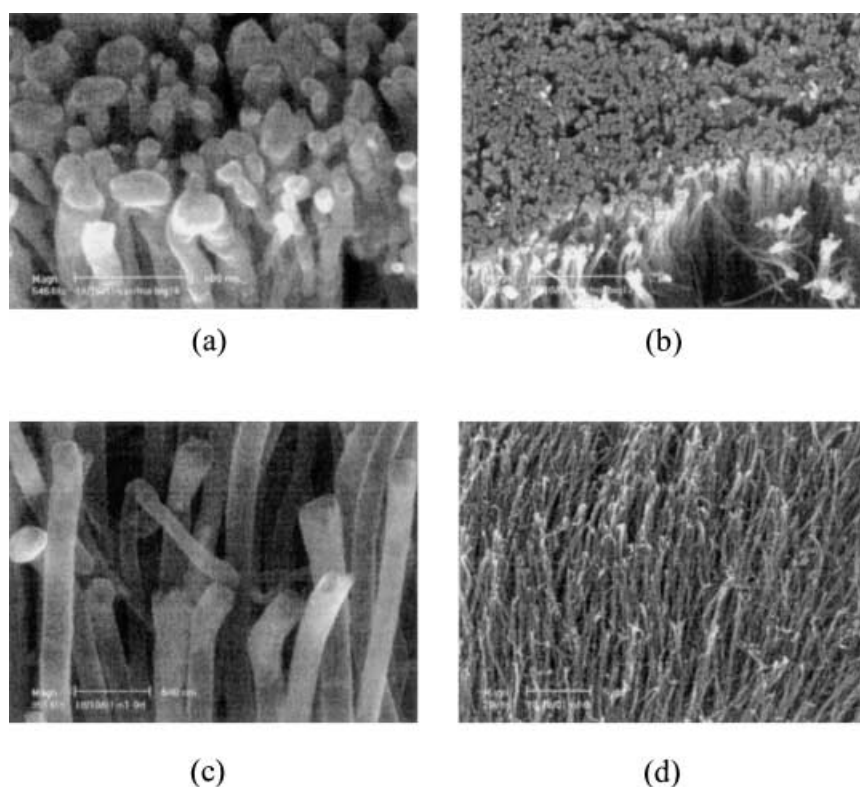
Fig. 2. SEM images of CNTs before (a, b) and after (c, d) oxidation in 37% HCl at 80 °C for 1 h. a) and c) at high magnification; b) and d) at low magnification, respectively. The alignment was remaining unchanged under this oxidation condition mainly depending upon the density of the sample employed.
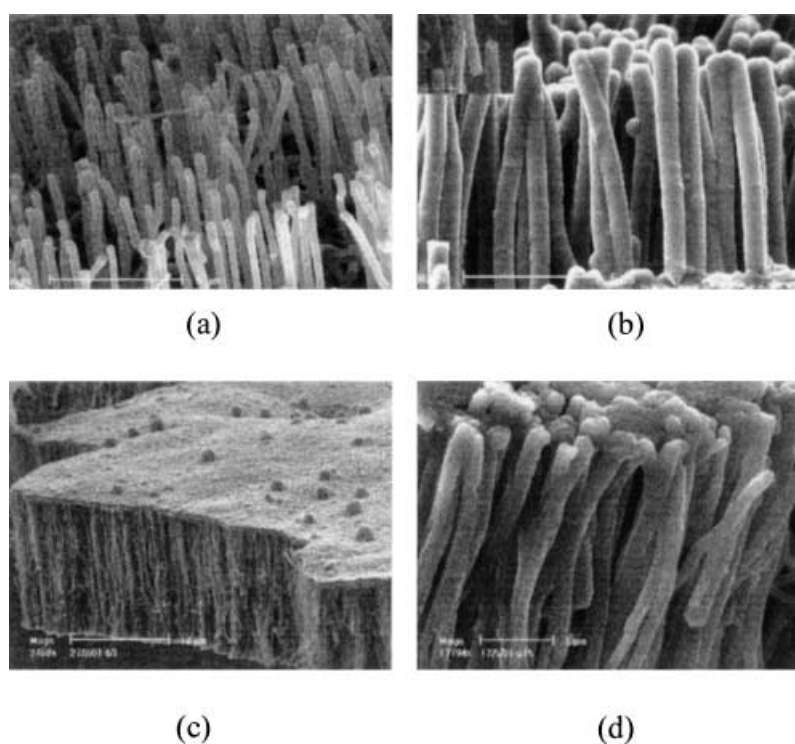

Fig. 3. SEM images of a) pure CNT array before PPy deposition; b) aligned PPy-CNT coaxial nanowires, inset shows clear image of single tube coated with PPY; c) PPy only deposited on the top of CNT surface due to the high density of the tube array; d) polymer formed on both outside of walls and the top of the surface of the CNT array. Potentiostatic oxidation (1 V) of 0.10 M pyrrole from an electrolyte solution containing 0.1 M NaClO4 in pH 7.45 buffer was used. Polymerization time was 1 min.

(Fig. 1a), much lower than the 0.65 V required to oxidize H_2O_2 on a gold electrode (Fig. 1b). The low oxidation potential obtained on the aligned CNT electrodes may be due to Fe particles present on the tips of the carbon nanotubes that are generated during the production of CNT mats (see Sec. 2). To investigate this, Fe particles were removed using acid solution (1 cm² of aligned CNT film was heated in 30% HCl at 80 °C for 1 h and the solution turned yellow due to Fe^{3+} generated). The sample was then rinsed with MilliQ water a few times until the wash solution was clear. The sample was dried in air for 4 hrs before recording SEM images, which show that the Fe particles sitting on the tips of individual carbon nanotubes prior to acid treatment have been removed (Fig. 2). After removing the iron particles it was found that the H_2O_2 response originally observed during cyclic voltammetry completely disappeared confirming that the iron nanoparticles present on the CNT tips do indeed play a critical role in lowering the oxidation potential for H_2O_2.

As a comparison, cyclic voltammograms of an entangled MWNT mat were recorded in the presence of H_2O_2. No oxidation responses were observed over the same potential range. XPS results show that the MWNT mat also contains iron. Hence, we conclude that both alignment and iron particles on the tube tips both contribute to the lowered oxidation potential observed for H_2O_2 at these novel CNT electrodes.

To ascertain the physical characteristics of the aligned carbon nanotube platform for efficient (coaxial) electrodeposition of the conducting polymer material polypyrroles containing just perchlorate as dopant were initially used. Potentiostatic oxidation was employed.

Scanning electron micrographs obtained after polypyrrole deposition (Fig. 3) revealed that the thickness of the polymer layer was less than 100 nm (Fig. 3b). An intertube spacing of 100 nm to 400 nm was required to allow efficient and even coating of the individual carbon nanotubes. If the tube density was too high (intertube spacing < 20 nm), polymer was deposited on the top of the mat rather than coating the individual tubes (Fig. 3c). With intertube spacing of 20 nm to 100 nm, the polymer was observed not only on the wall of the tubes, but also on the top of the mat (Fig. 3d).

Initial attempts at glucose immobilization involved simple adsorption experiments on bare CNT electrodes. This was unsuccessful. Subsequently a conducting polymer-GOX polymer was electrodeposited using a 0.10 M $NaClO_4$ containing electrolyte with GOX (2 mg/mL) present. To determine the optimal potential for glucose detection on the PPy/GOX/CNT electrode, aligned CNT electrodes with uniform PPy coatings on each of the individual nanotubes were prepared as described in the experimental section. The current-time transient observed during polymerization indicates that a conducting polymer was deposited (Fig. 4). Following the initial charging spike a rising current transient is indicative of conducting polymer deposition. After approximately 5 seconds the current started to decrease presumably due to depletion of the monomer at this high
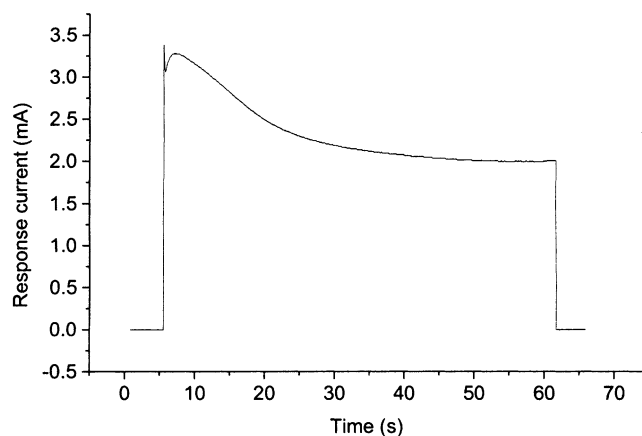
Fig. 4. Polymerization conditions as for Figure 3 with 2 mg/mL GOX added in the monomer solution at 10 °C. An aligned carbon nanotube array (0.09 cm²) was used as the working electrode. The counter electrode was Pt foil (1 cm²) and the reference electrode was Ag/AgCl.
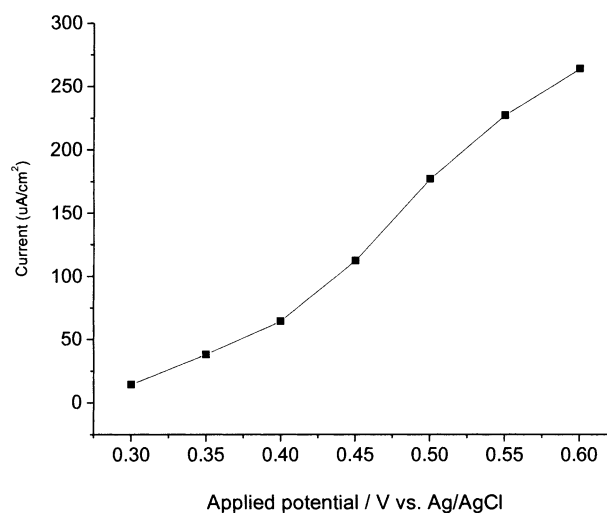

Fig. 5. Effect of the applied potential on the response current of the PPy/GOX/CNT electrode to 20 mM glucose in 0.10 M buffer solutions (pH 7.45).

surface area electrode. The polymer thickness on the individual carbon nanotube can be controlled by the amount of charge passed, during polymerization.

Using the polymer coated aligned carbon nanotube structures obtained by polymerization in the presence of GOX the response to glucose addition was investigated. This was achieved using a chronoamperometric set up at different applied potentials.

It was found that the current obtained upon addition of glucose increased sharply at potentials between 0.40 to 0.55 V (Fig. 5). This concurs with the results obtained during cyclic voltammetry of hydrogen peroxide on PPy/CNT electrodes, where the oxidation peak appeared at ca. 0.45 V (Fig. 6). Although there is a potential shift from that observed with bare aligned carbon nanotube arrays, the potential required for H_2O_2 oxidation was still relatively low
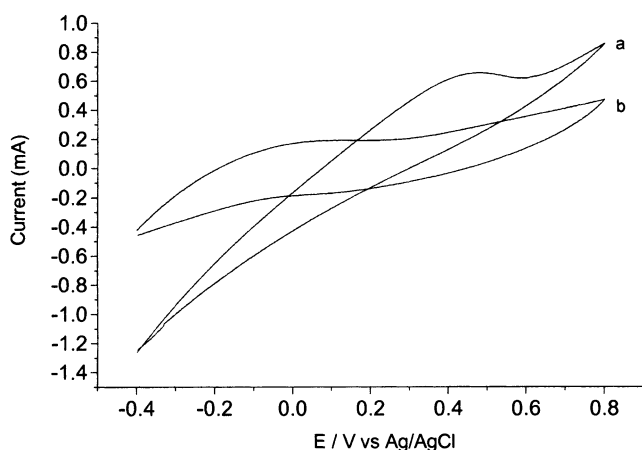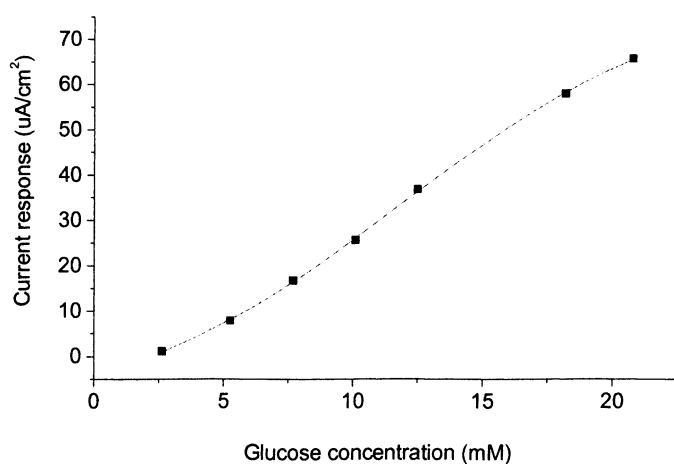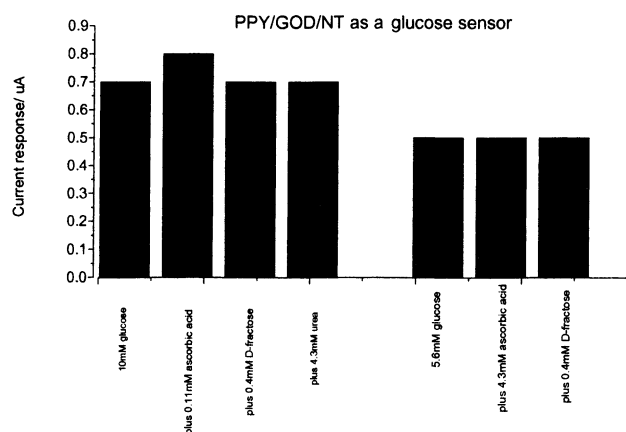
Fig. 6. Cyclic voltammograms obtained for a) at PPy/CNT electrode in 0.10 M H₂O₂/pH 7.45 buffer solution (0.10 M); b) at PPy-GOX coated CNT electrode in buffer only. as prepared in Figure 4.

(a)

(b)

Fig. 7. a) Dependence of anodic current of PPy/GOX/CNT electrode on glucose concentration at 0.45 V in 0.10 M phosphate buffer (pH 7.45); b) Influence of interferent reagents on glucose response current in 0.10 M buffer solution (pH 7.45) after adding biological level of ascorbic acid (0.11 mM), D-fructose (0.4 mM) and urea (4.3 mM) to two glucose concentrations of 5 mM and 10 mM.

compared to those reported previously in the literature for simple polypyrrole-GOX films [23].

The effect of glucose concentration on the response obtained at PPy/GOX/CNT electrodes was investigated. As can be seen in Fig. 7a, the response of the electrode to glucose increased with an increase of the glucose concentration. The linear relationship is up to 20 mM, which is higher than the 15 mM required for practical use in the detection of blood glucose. Comparison of results obtained using a gold electrode substrate (PPy/GOX/gold) versus the CNT array (PPy/GOX/CNT) shows that responses obtained with the latter were at least an order of magnitude greater. This is despite the fact that the gold electrode in this comparison contains 0.08 mg/cm² polymer compared with 0.05 mg/cm² on CNT as confirmed by thermal gravimetric analyses. The influence of potential interferents such as ascorbic acid, urea, and D-fructose on the CNT based sensor was investigated (Fig. 7b). The results show that interference levels were low or negligible. The ability to use low potentials for H₂O₂ oxidation results in this improved selectivity.

This sensor retains its stability at 70% after 3 days storage in the dry state at 4 °C.

4. Conclusions

The use of aligned carbon nanotube arrays with iron particles on the CNT tips have been shown to be useful as a platform on which to deposit PPy with GOD entrapped. Using this polymer coated structure here sensitivity and high selectivity towards glucose has been demonstrated. The role of iron particles, the catalysts responsible for the tube growth, together with the alignment of the tubes are shown to be the key factors in the lower potential for H₂O₂ oxidation.
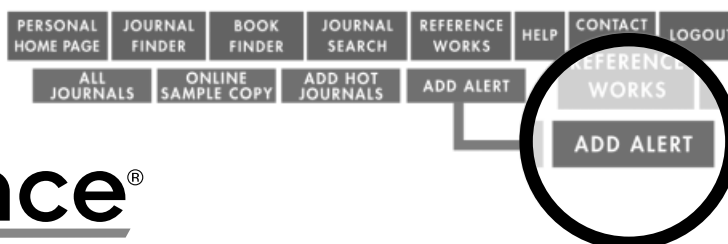
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