

Major Achievements in the Analytical Chemistry of Antioxidants and Explosives

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The widely used and cited CUPRAC (cupric ion reducing antioxidant capacity) method was originally developed in our laboratory as an electron transfer-based assay, but later evolved into an integrated series of measurements for antioxidant characterization, such as the measurement of reactive species (i.e. H_2O_2 , hydroxyl and superoxide radicals) and enzymes where either the original probe or the converted product showed CUPRAC

reactivity. A low-cost colorimetric antioxidant sensor (CUPRAC sensor) working like a pH test-strip was developed by immobilizing the Cu(II)-Nc reagent onto (i) a Nafion perfluorosulfonate cation-exchange polymer membrane, and (ii) a polysulfate natural polymer, carrageenan membrane. DMPD (*N,N*-dimethyl-*p*-phenylenediamine) sensor could measure both oxidants and antioxidants on the same Nafion membrane. DNA was first damaged



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by reactive oxygen species (ROS) to give a colored CUPRAC product, which was in turn decreased with the addition of antioxidants. Energetic substances in our analysis portfolio include explosives, pyrotechnics and propellants, and their prompt detection is important for the safety and well-being of modern societies exposed to various security threats. The incorporation of nanotechnology has greatly contributed to colorimetric sensor design due to the superb physico-chemical properties of nanomaterials, including the effects of size, geometry, specific surface area, surface plasmon resonance (SPR) absorption and nanozyme catalysis. As for possible mechanisms of analyte detection with colorimetric/fluorometric sensors, a wide spectrum of physico-chemical interactions between analytes and nanoprobe may be significant depending on the purpose, such as (photo)induced electron transfer, H-atom transfer, charge transfer, radical scavenging, donor-acceptor, Lewis acid-base neutralization, electrostatic, dipole-dipole, π - π stacking, ion- π , hydrogen bonding and supramolecular interactions, which almost comprise all aspects of the electromagnetic force, standing out among the four essential forces of the universe exploitable in analytical applications. Optical nanosensors and nanoprobe have been treated in terms of their mechanisms of operation, *i.e.* formation and growth of noble metal nanoparticles including derivatized nanoparticle-analyte binding, aggregation/disaggregation of nanoparticles, displacement of active constituents by complexation or electrostatic interaction, miscellaneous mechanisms, and the choice of metallic oxide nanoparticles taking part in such formulations. Colorimetric sensors may be more useful for fast decision making and screening analyses in on-site applications, while electrochemical sensors generally give a more reliable estimate of explosive traces remaining in crime scenes and contaminated/remediated soil. Application of 2-D materials to electropolymerized sensor electrodes comprising imprinted polymers has greatly increased the sensitivity and selectivity of explosives trace estimation. Thus, our milestones in sensor design for antioxidants and energetics have been presented with examples and related mechanisms.



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1. Measurement of Antioxidant Activity and Capacity

In a simple and understandable term, antioxidant (AOX) is a substance inhibiting or delaying undesired oxidation reactions. An antioxidant is technically defined as 'any substance in low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of the substrate' (Gutteridge, 1994). Antioxidants have been traditionally divided into two classes; primary or chain-breaking antioxidants, and secondary or preventative antioxidants. One important function of antioxidants toward free radicals such as hydroxyl, superoxide anion or peroxy radicals *etc.* is to extinguish free radical-mediated oxidation by inhibiting the formation of free radicals and/or by scavenging radicals. Antioxidants are secondary metabolites that contain bioactive substances, phytonutrients, nutraceuticals mainly produced by the plants. They present a wide range of health-promoting properties, in that *in vitro/in vivo* studies have shown that dietary intake of antioxidant-rich foods may reduce the risk of many oxidative stress-originated diseases such as some types of cancer, Alzheimer, diabetes, cardiovascular and inflammatory complications, aging and obesity (Cheung et al. 2015).

In monitoring food quality and nutrition, antioxidants are analyzed for the distinguishing comparison of foods regarding their antioxidant content and screening geographical or seasonal variations within or between complex food products. The chemical diversity of antioxidants complicate to separate and quantify individual antioxidants (*e.g.*, polyphenols, their glycosides, and their many isomers) from the vegetable matrix (Çelik et al., 2010a). Total antioxidant capacity is a more meaningful indicator of the collaborative (and usually additive) behavior of all antioxidants present in a complex sample, enabling it to be recognized by leading researchers as a more useful parameter to evaluate health beneficial effects of food/plasma antioxidants (Apak, 2019). Researchers are still after efforts to develop and standardize methods to measure the antioxidant abilities of plant-based food extracts and biological fluids because of the lack of a widely accepted total antioxidant activity/capacity parameter as a reference nutritional index for food and biological fluid labeling (Apak et al., 2022).

Cupric ion reducing antioxidant capacity method is a simple, versatile, selective, low-cost assay for all types of biologically important antioxidants (*e.g.*, ascorbic acid, α -tocopherol, β -carotene, reduced glutathione (GSH), uric acid, and bilirubin) and all types of food antioxidants (*e.g.*, flavonoids, simple phenolic and hydroxycinnamic acids)

regardless of chemical type or hydrophilicity. The light blue-colored bis(neocuproine) copper(II) complex was used as the chromogenic redox reagent. As a result of redox reaction with reducing polyphenols at pH 7, the absorbance of the yellow colored Cu(I)-chelate was measured at 450 nm (within 30 min for the slowest reacting antioxidants). The cupric reducing antioxidant capacity method was introduced by our research group and abbreviated as the 'CUPRAC' method (Apak et al., 2004) (Figure 1). This is an electron-transfer based colorimetric assay that measures the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change is correlated to the concentration of antioxidants in the sample (Apak et al., 2008). The cupric/cuprous redox couple in the presence of neocuproine ligand assumes the most appropriate potential of 0.60 V capable of oxidizing most biological and food antioxidants (compared to the 0.17 V potential of $\text{Cu}^{2+}/\text{Cu}^+$ couple), because the molecular strain is relieved in the perfectly tetrahedral cuprous-neocuproine chelate relative to the cupric-neocuproine complex distorted by Jahn-Teller effect, thereby preferentially stabilizing the cuprous state and raising the standard potential, as seen below:

The CUPRAC method has been successfully applied world-wide in research laboratories using standard equipment like a colorimeter rather than more expensive

and sophisticated instrumental techniques necessitating qualified operators. The CUPRAC reagent is much more stable and easily accessible than the conventional chromogenic radical reagents (*e.g.*, ABTS, DPPH), added to the fact that it gives a linear response over a wide concentration range thanks to the generation of a single chromophore with antioxidants, *i.e.* cuprous-neocuproine. CUPRAC method was successively adapted to different types of complex matrices, *i.e.*, herbal plants (Apak et al., 2006; Güçlü et al., 2006; Yıldız et al., 2008; Alpınar et al. 2009; Çelik et al. 2014; Çelik et al. 2017), human serum (Apak et al. 2005), fruit and vegetable samples (Çelik et al. 2012, Bener et al. 2022a, Karaman et al. 2010), cereals (Tufan et al., 2013), edible oils (Çelik et al. 2019a; Altun et al. 2013). The two leading CUPRAC papers have retrieved over 4000 citations, and many more with CUPRAC modifications. From the parent CUPRAC method, a number of "daughter" methods have developed (Özyürek et al. 2007; Özyürek et al. 2008a; Çelik et al. 2007, Çelik et al. 2010a; Tufan et al. 2014). CUPRAC method has several advantages over similar electron transfer-based methods in terms of selectivity, precision and accuracy. CUPRAC users worldwide shared their experiences and showed important advantages of this assay (Prior et al. 2005; Gorinstein et al. 2006). Copper complexed with 2,9-dimethyl-1,10-phenanthroline has a lower redox potential than iron, so its reactions are more selective. The comparatively

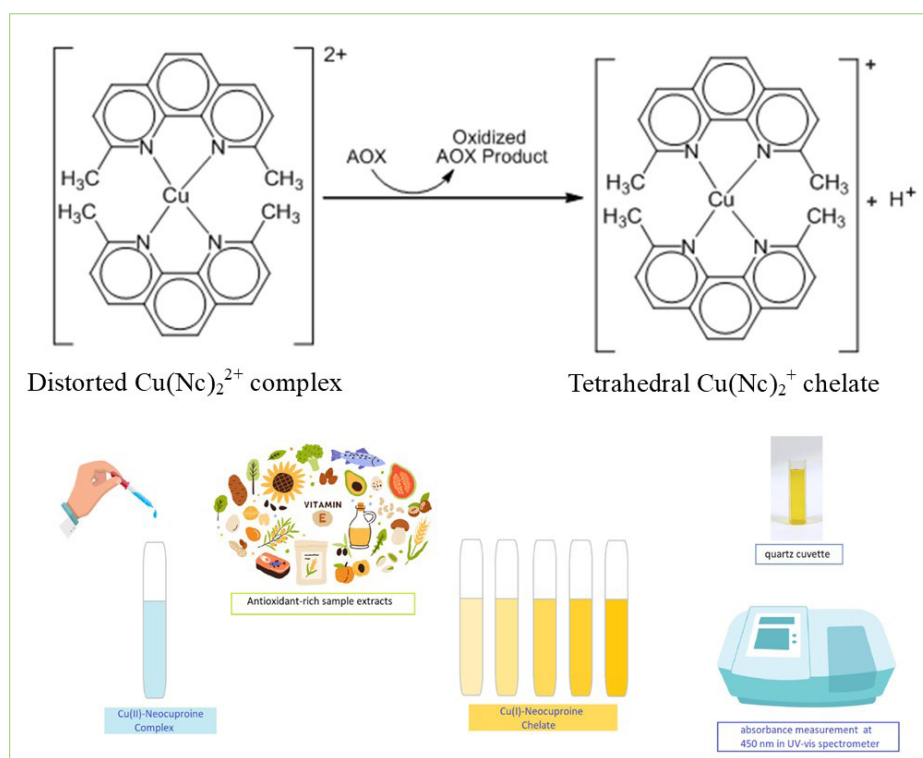


Figure 1. Schematic representation of the CUPRAC assay.

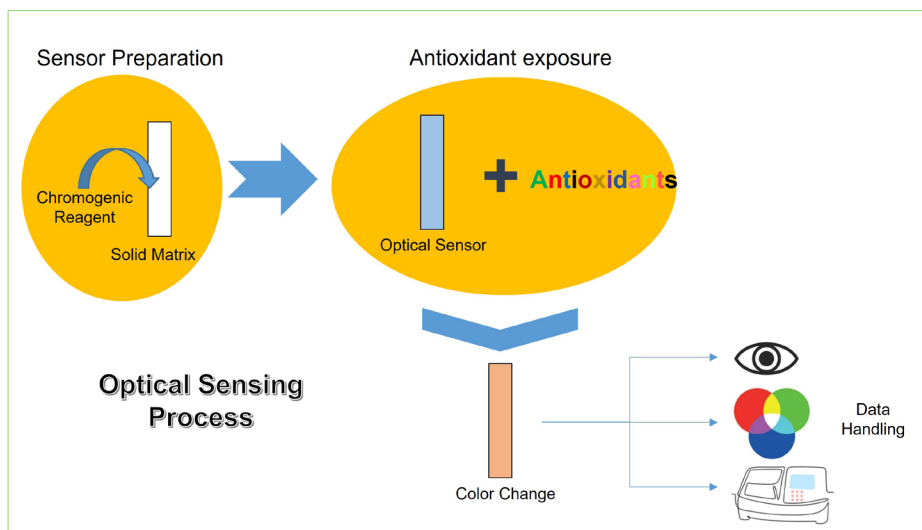


Figure 2. Optical sensing process for antioxidants on suitable membranes.

low redox potential of Cu(II)-Nc/Cu(I)-Nc (0.60 V) make it easier for antioxidant compounds to be oxidized; simple sugars and citric acid mainly found in food samples, which are not true antioxidants, are not oxidized with the CUPRAC reagent. Precision (intra- and inter-day RSD%) are definitely better than those of most kinetic-based assays. The method can simultaneously measure hydrophilic and lipophilic antioxidants (e.g., β -carotene and α -tocopherol). The CUPRAC redox reaction is relatively insensitive to air oxygen, sunlight, humidity, and pH within a reasonable range. The method is also suitable for automation (Apak et al. 2008). CUPRAC proved to be relatively independent of solvent effects in alcohol-water mixtures of varying compositions so as to be useful in different plant extracts (Çelik et al., 2010b).

1.1. Colorimetric sensors and nanoprobes for antioxidants

In recent years, the use of colorimetric sensors and nanoprobes that provide detection with the naked eye has come to the fore for the field/*in situ* detection of important bioactive components such as antioxidants. Moreover, using nanosensors brings significant advantages to this field due to its various physico-chemical advantages such as increased surface area, surface plasmon resonance absorption of noble metal nanoparticles, and superior enzyme-mimic catalytic properties. General sensing principles of nanomaterials are based on the formation, growth, aggregation, disaggregation, and etching of nanoparticles giving rise to distinct spectral variations. Optical- and nano-sensor based methods have been developed by our working group to detect antioxidants.

In the first study of this concept, a low-cost optical antioxidant sensor (CUPRAC

sensor) was developed by immobilizing the copper(II)-neocuproine (Cu(II)-Nc) reagent on Nafion, a cation-exchange polymer membrane (Bener et al., 2010). Highly colored Cu(I)-Nc chelate is formed on the membrane due to the reaction of antioxidants with immobilized Cu(II)-Nc reagent, and the absorbance changes associated with this color change are measured at 450 nm. In another study, TAC determination was performed by measuring the reflectance changes on the membrane at 530 nm, adhering to the same preparation procedure as the previously developed CUPRAC method (Bener et al., 2013). TAC values of commercial fruit juices and tissue homogenates were determined using this reflectometric sensor, and the sensor was successfully applied to turbid samples. Alternatively, a biopolymer (carrageenan polysaccharide) film was used instead of Nafion, adhering to the same detection mechanism as the CUPRAC sensor (Bener et al., 2018a). The cationic Cu(II)-Nc reagent was immobilized on the biopolymer film owing to the anionic sulfate groups of carrageenan. The biopolymer film chosen as the support material costs 95% lower than the Nafion film, and the biopolymer is environmentally friendly with its biodegradable structure. Another TAC optical sensor on Nafion support material is based on the *o*-phenanthroline method (Bener et al., 2017). Fe(III)-*o*-phenanthroline (Fe(III)-phen) reagent immobilized on the membrane is reduced to orange-red Fe(II)-phen in the presence of antioxidants and the absorbance change is recorded at 510 nm. Optical sensors developed using both Nafion and biopolymer film support materials for TAC measurement are not particularly affected by potential interferents from colored or turbid solutions such as food samples. In addition, these types of optical sensors are easily convertible to

kit format for on-site TAC analysis (Figure 2). In another study, a colorimetric sensor was developed that simultaneously measures the oxidative status formed by reactive oxygen species (ROS) and the antioxidant activities of ROS-scavenger compounds (Çekiç et al., 2015). After ROS generation, the Nafion membrane surface was colored pink due to cationic quinone radicals formed by the oxidation of *N,N*-dimethyl-*p*-phenylene diamine (DMPD), but this color was partly bleached with ROS-scavengers where absorbance changes were recorded at 415 nm.

The “Silver Nanoparticle Antioxidant Capacity” (SNPAC) test, based on the formation and growth mechanism of nanoparticles, measured the Ag⁺ ion reduction ability of polyphenols (Özyürek et al., 2012a). The reduction of Ag⁺ to spherical silver nanoparticles (SNPs) by polyphenols in the presence of trisodium citrate and silver seeds produces a very intense surface plasmon resonance (SPR) absorption band at 423 nm. SNP seeds were coated with nascent nanoparticle layers in a controlled manner by adding polyphenolic antioxidants for total antioxidant capacity (TAC) measurement. In our department, a green silver nanoparticle based antioxidant capacity (GSNP-AC) method using carob extract for the formation of AgNPs was developed for TAC measurement (Beğiç et al., 2021). These AgNPs have several advantages such as green synthesis, environmental friendliness, reliability, sustainability and rapid production. In the presence of Ag seeds formed by carob extract, Ag⁺ is reduced to spherical silver nanoparticles by antioxidants and shows a symmetrical SPR absorption band at 434 nm, not significantly shifted by AOX addition. The SPR absorption of AgNPs showed a linear increase in absorbance depending on the amount of antioxidants (Figure 3). In another study on sensor development based on nanoparticles formation and growth for TAC measurement, κ -carrageenan (CAR) was used for the green synthesis of AgNPs (Öztürk et al., 2021). Silver ion (Ag⁺) was reduced by antioxidants to spherical SNPs around the initially formed κ -carrageenan capped silver nanoparticles (CAR-SNP). After the reaction with antioxidants, the symmetrically enlarged AgNPs exhibited a SPR absorption band at 420 nm having linear dependence on AOX concentration. The higher sensitivity achieved in TAC measurement compared to other similar methods based on the same mechanism arises from the fact that the negatively-charged ester sulfate groups of CAR on functionalized AgNPs attract more Ag⁺ ions capable of oxidizing phenolic antioxidants with higher efficiency.

A sensitive and rapid nanosensor was developed in our department to detect

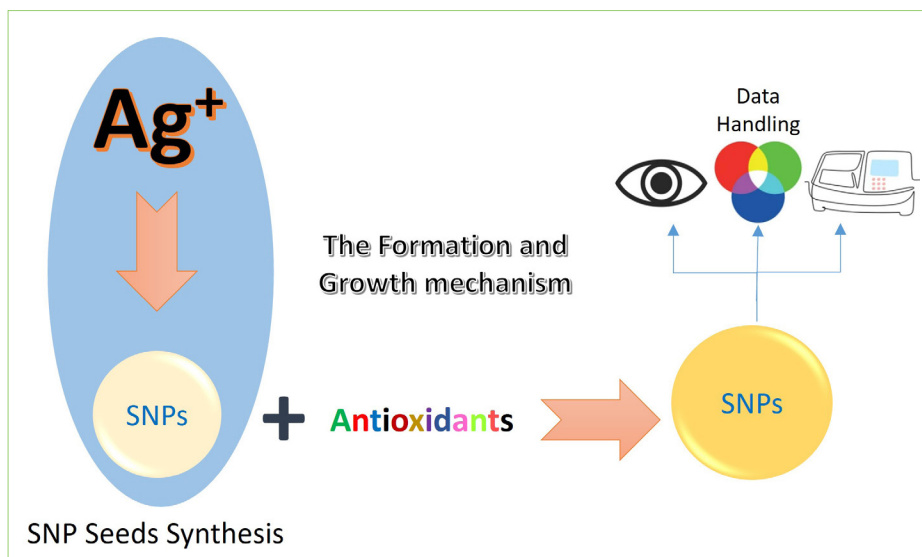


Figure 3. Antioxidant determination based on the formation and growth mechanisms of silver nanoparticles (SNPs) used as nanosensors.

thiol-type antioxidants by functionalizing gold nanoparticles (AuNPs) with Ellman thiol reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB) (Güçlü et al., 2013). AuNPs were synthesized by the classical citrate reduction method and derivatized with DTNB. DTNB was bound to AuNPs possibly through the S-S linkage, and absorbance changes associated with the formation of yellow 5-thio-2-nitrobenzoate (TNB²⁻) anion by reaction with thiol-type antioxidants were recorded at 410 nm. By using the enhanced selectivity/sensitivity of thiol-exchange reaction of the DTNB-AuNP probe, biothiols could be distinguished from other antioxidants. In another study, we developed a CUPRAC colorimetric sensor based on heparin-stabilized AuNPs (Bener et al., 2018b). Negatively-charged AuNPs stabilized with heparin were added to the Cu(II)-Nc solution resulting from the reaction of Cu(II)-Nc with antioxidants. The absorbance of Cu(II)-Nc-AuNPs developed by electrostatic adsorption of Cu(II)-Nc cationic chelate on heparinated gold nanoparticles was measured at 455 nm. This colorimetric sensor retained the traditional CUPRAC selectivity for antioxidant detection while maintaining the advantage of a nanosensor with significant inert electrolyte tolerance without aggregation at almost 1000-fold levels of ordinary salts, thanks to the extreme negative charge density of heparin. This method accelerated the oxidation of a compound such as thiourea, which normally reacts slowly with the CUPRAC reagent in the solution phase, due to thiourea's affinity toward the gold surface. In addition, this nanosensor also serves green analytical chemistry, thanks to both the use of heparin as the reducing/stabilizing agent for AuNP synthesis and the significantly reduced reagent consumption for TAC determination. A similar concept

to antioxidant activity is prooxidant activity, related to the ability of test compounds to generate new reactive species that can damage biomacromolecules. For the measurement of Cu(II)-induced prooxidant activity of natural antioxidants, a chicken egg white protein-protected gold nanocluster (CEW-AuNC)-based fluorogenic biosensor was developed in our department (Akyüz et al., 2020). Proteins in chicken egg white functioned as both reducer and preservative in the preparation of AuNCs. The proposed fluorometric method is manifested with a large Stokes shift (excitation/emission at 360/640 nm). The fluorescent intensity of the biosensor was attenuated with common antioxidants that could eventually reduce Cu(II) to Cu(I), eventually retained by the protein -SH groups on the nanocluster. Since Cu(I) can generate reactive oxygen species *in situ*, the copper(II) reducing ability of the test compounds indicated their prooxidant activities. The proposed sensor could determine the Cu(II)-derived total prooxidant activity (TPA) of natural antioxidants abundant in food and biological samples in the mM range. The biosensor involved one-pot biosynthesis and was the first of its kind to combine AuNCs with the perfectly linear response of fluorescence intensity. In another study using the same probe, a spectrophotometric method was developed using the CEW-AuNC biosensor to measure the Cu(II)-induced prooxidant activity of natural antioxidants (Akyüz et al., 2019).

1.2. Electrochemical sensor electrodes for antioxidant assays

Studies have also been carried out in our department to determine antioxidants with electroanalytical sensors. A differential pulse voltammetric (DPV) method using the chromogenic oxidizing reagent,

cupric-neocuproine complex (Cu(II)-Nc), was developed to evaluate the antioxidant capacity of polyphenolic compounds, ascorbic acid and real samples (Tufan et al., 2014). The electrochemical behavior of the Cu(II)-Nc complex was investigated by cyclic voltammetry on a glassy carbon electrode (GCE). The electroanalytical method is based on the reduction of Cu(II)-Nc to Cu(I)-Nc by antioxidants and measuring the reduction current of the remaining Cu(II)-Nc (unreacted complex). The reagent on GCE retained its reactivity to antioxidants, and the measured trolox equivalent antioxidant capacity (TEAC) values of various antioxidants were comparable to the solution-based spectrophotometric CUPRAC results. In this regard, Ayaz et al. (2022) developed an even more sensitive electrochemical assay for amperometric TAC estimation in conjunction with flow injection analysis (FIA) using Cu(II)-Nc complex modified on Nafion-functionalized multi-walled carbon nanotube (MWCNT)-glassy carbon electrode (GCE). The enhanced sensitivity (i.e. lower LOD for antioxidants) of this novel electrode for TAC determination resulted from a combination of favorable properties such as MWCNTs modification of GCE, electrostatic retention of the cationic Cu(II)-Nc chelate on Nafion sulfonate groups, and measurement of the anodic oxidation current of antioxidant-reduced Cu(I)-Nc chelate (rather than of the reduction current of Cu(II)-Nc, which is measured against an intense background signal). Another electroanalytical TAC sensor developed in our department is based on the chemical reduction of hexacyanoferrate(III) to hexacyanoferrate(II) by antioxidants, monitored by the attenuation of cathodic current intensity of [Fe(CN)₆]³⁻ in proportion to antioxidant concentration (Arman et al., 2019). During voltammetric measurements, the surface of the GCE was coated with an *o*-phenylenediamine-aniline copolymer, and AuNPs were deposited on this electrode surface to increase conductivity. It was shown that the developed electrode gives a reversible voltammogram for the hexacyanoferrate(III)/(II) redox couple, and cathodic peak decrements occur in the presence of strong antioxidants with standard redox potentials lower than that of this redox couple ($E^{\circ} < 0.36$ V). This voltammetric sensor allowed sensitive measurement of the total antioxidant capacity of herbal tea samples.

2. Measurement of reactive oxygen and nitrogen species (ROS/RNS) and their scavengers

Reactive oxygen and nitrogen species (RONS) are either free radicals (hydroxyl radical, superoxide anion radical, peroxy radical, nitric oxide radical) or reactive molecules/ions (hydrogen peroxide, hypochlorous acid, peroxyxynitrite anion) derived from

molecular oxygen and nitrogen species. They are required for normal cell functions such as intracellular signaling and cellular defense against infective agents, but their excessive production can damage important biomolecules by taking part in the pathogenesis of various diseases, including cardiovascular diseases and cancer. When the balance between RONS and intrinsic/extrinsic antioxidants cannot be maintained by redox reactions between the two chemical species, the cellular environment becomes oxidatively stressed (Figure 4) (Apak et al., 2016).

Detection and characterisation of radicals is accomplished by using electron paramagnetic resonance (EPR) spin trapping and related techniques. There are also costly and laborious techniques that combine chromatography with mass spectrometry (LC-MS/MS) for detecting RONS. Attempts to evaluate RONS production and pro-oxidant/antioxidant balance often rely on biomarkers of oxidative stress through *in vitro* experiments. The most preferred approach is an indirect detection of ROS via detection of products of their reactions (e.g., with the use of spectroscopic probes) because of their brief half-life and low steady-state concentrations under physiological conditions (Bartosz, 2006). A spectroscopic probe is a substance that, when reacted with ROS, can change its spectral properties such as light absorption or emission. Recently, nanoparticles have been used in the detection of ROS and the determination of antioxidant activity based on scavenging of these reactive species due to their unique physico-chemical properties (Kaboggoza et al., 2023).

The hydroxyl radical ($\bullet\text{OH}$) is one of the strongest ROS that can react with almost all substances. In the literature, $\bullet\text{OH}$ determination, which is usually produced by the Fenton reaction ($\text{Fe(II)} + \text{H}_2\text{O}_2$), is performed using the thiobarbituric acid-reactive substances (TBARS) test (Halliwell & Gutteridge, 1981) which has been criticised due many interferences producing false-positives. In the presence of $\bullet\text{OH}$, aromatic hydroxylation products of spectroscopic probes such as salicylate and terephthalate can be detected by the CUPRAC assay (Özyürek et al., 2008b). Due to the molecular symmetry of terephthalate, a single hydroxylation product (2-hydroxy terephthalate) was formed as a result of the reaction with $\bullet\text{OH}$, which was in turn determined using the CUPRAC colorimetric sensor on a Nafion membrane (Bekdeşer et al., 2012).

The superoxide anion radical ($\text{O}_2^{\bullet-}$) can be produced in various biological systems either by autooxidative processes, by enzyme-catalyzed (xanthine-xanthine oxidase) (Terada

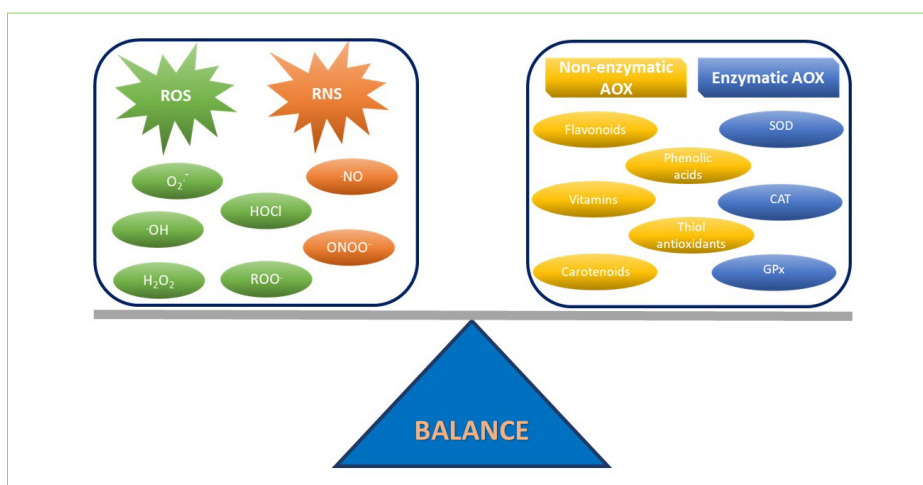


Figure 4. Balance between reactive species (ROS and RNS) and antioxidants.

et al., 1990) or non-enzymatic reactions (phenazine methosulfate-nicotinamide adenine dinucleotide) (Unno et al., 2002). The nitroblue tetrazolium (NBT) method is often used to determine the superoxide radical scavenging activity of antioxidant compounds (Bielski et al., 1980), but it suffers from problems arising from formazan solubility. Bekdeşer et al. (2011) used *tert*-butylhydroquinone (TBHQ) probe for detecting superoxide anion radical and superoxide dismutase enzyme activity. In this method, TBHQ was considered as a superior probe in comparison to existing probes (NBT and Cyt c) in terms of selectivity (Bekdeşer et al., 2011).

Hydrogen peroxide can be determined spectrophotometrically with the aid of its UV light absorption at 230 nm or of peroxidotitanate (IV) complex formation with titanium (IV) (Beers & Sizer, 1952; Sellers, 1980). The UV method is prone to interference by plant pigments absorbing light in the UV-region. As an alternative to numerous methods, the H_2O_2 scavenging activity of polyphenols was quantified by direct measurement of undegraded H_2O_2 concentration using the CUPRAC method in the presence of a Cu(II) catalyst (Özyürek et al., 2010). Similarly, catalase activity was indirectly determined by using the reaction of Cu(II)-Nc reagent electrostatically immobilized on the Nafion membrane, through measurement of undegraded hydrogen peroxide remaining in the reaction medium after annihilation with catalase (Bekdeşer et al., 2014). The proposed methodology is both free from interference effects in the UV region and is superior to rather non-specific horseradish peroxidase-based assays. Üzer et al. (2017a) developed an indirect method for the determination of trace H_2O_2 (LOD \approx 20 nM) by measuring the absorbance resulting from TMB oxidation with Ag^+ formed as a result of the catalytic reduction of H_2O_2 by silver nanoparticles.

Peroxy radicals (ROO^{\bullet}), which play an important role in the chain progression step of lipid peroxidation, are generally produced by thermal decomposition of 2,2'-azobis(2-methylpropionamide) dihydrochloride. The oxygen radical absorbance capacity (ORAC) assay is the most popular assay for the extent of inhibition of ROO^{\bullet} -induced oxidation. In the presence of ROO^{\bullet} , the fluorescence signal of the probe (*i.e.* β -phycoerythrin (Cao et al., 1993) or fluorescein (Ou et al., 2001)) decreases. As an alternative to these molecules, Güçlü et al. (2014) developed a new fluorimetric probe for ROO^{\bullet} using *p*-aminobenzoic acid, which also responded to hypochlorous acid (She et al., 1997). Çelik et al. (2019b) used starch-based gold nanoparticles (AuNPs) for indirect determination of antioxidant activity. Peroxy radicals oxidized iodide ions to triiodide, which was rapidly adsorbed on the surface of AuNPs, causing their aggregation. In the presence of antioxidants, the red-shifted surface plasmon resonance absorption of the aggregated starch-stabilized AuNPs decreased. When GSH-capped gold nanoclusters (AuNCs) were used as fluorescent probe and AAPH-induced oxidation of GSH occurred, the initial fluorescence intensity of GSH-capped AuNCs ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 502 \text{ nm}$) was decreased with static quenching (Kaboggoza et al., 2023).

Hypochlorous acid (HOCl) and hypobromous acid (HOBr) are strong oxidants produced in neutrophils through the reaction of chloride or bromide ions with H_2O_2 , catalyzed by myeloperoxidase, respectively. The TNB (2-nitro-5-thiobenzoate)/DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) assay, which is frequently used for the determination of HOCl and HOBr scavenging activity, is open to interferences due to the fact that biothiols directly reduce DTNB to TNB. Resorcinol (Özyürek et al., 2012b) and *p*-c-resol (Bekdeşer et al., 2018) were used as fluorogenic probes to determine the HOCl

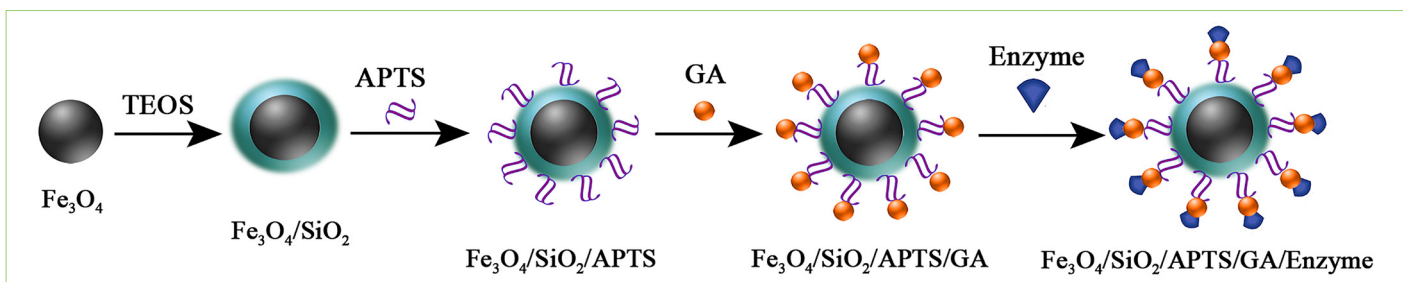


Figure 5. The proposed procedure for covalent attachment of selected oxidase enzymes on the modified Fe_3O_4 MNPs.

and HOBr scavenging activity of antioxidants, respectively.

Nitric oxide radical ($\cdot\text{NO}$), a biochemically important stable radical, generated from nitroprusside system can be spectrophotometrically measured with the Griess reagent, after conversion to nitrite (Green et al., 1982). Can et al. (2022) developed a nanoparticle-based method in which $\cdot\text{NO}$ was converted to nitrite, and the azo dye formed with the coupling-agent naphthyl ethylenediamine (NED) in the presence of 4-aminothiophenol (ATP)-modified gold nanoparticles was determined using its 565 nm absorption.

3. *In vitro* determinations in biological and food samples using natural enzymes

3.1. Immobilization of selected oxidase enzymes and determinations of their substrates

Enzymes are vital protein molecules that help to accelerate various physiological reactions. Enzymes can be immobilized on inert surfaces, especially on nanomaterials, for reuse over extended periods and easier separation from reaction products during *in vitro* studies. In this regard, Avan et al. (2022) synthesized Fe_3O_4 magnetic nanoparticles (MNPs) by coprecipitation of Fe(II) and Fe(III) salts in order to obtain a solid support for immobilization of three different oxidase enzymes, namely uricase (UOx), glucose oxidase (GOx), and choline oxidase (ChOx), separately. These oxidases were attached to the surface of MNPs covalently; the surface of MNPs was modified by consecutive application of tetraethyl orthosilicate

(TEOS), (3-aminopropyl) triethoxysilane (APTS) and glutaraldehyde (GA). Finally, the oxidase enzymes UOx, GOx and ChOx were dissolved in appropriate buffer solutions separately, then added onto $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{APTS}/\text{GA}$ modified nanoparticles and mixed (Figure 5). Thus, the fabricated colorimetric nanobiosensors were used to determine the oxidase enzyme substrates, namely, uric acid (UA), glucose (Glu) and choline chloride (Ch), in food and biological samples.

The enzyme-immobilized MNPs were characterized by scanning transmission electron microscopy (STEM), energy-dispersive X-ray spectrometry (EDS) and Fourier transform infrared spectroscopy (FTIR). The fabricated nanobiosensors were used for quantification of Glu, Ch, UA, and also UA-free total antioxidant capacity (TAC) with the use of colorimetric CUPRAC method. The released H_2O_2 from the immobilized enzyme-substrate interactions was absorbance detected with the CUPRAC reaction at 450 nm. For determination of UA-free TAC, another enzyme, catalase, was added to the reaction medium containing UA (or UA containing antioxidant mixture) and UOx@MNPs, because both UA and the generated H_2O_2 were CUPRAC-responsive. Hence, the generated H_2O_2 was eliminated by catalase, and the CUPRAC absorbance before and after treatment of UOx were measured. The difference between these two-absorbance values (ΔA) showed the UA and UA-free TAC in antioxidant mixtures. The proposed procedure, summarized in Figure 6, was applied to real samples.

3.2. Determination of fish freshness with the use of CUPRAC colorimetric sensor applied to hypoxanthine-xanthine oxidase system

As soon as a living organism dies, the biosynthesis of adenosine triphosphate (ATP), the main source of cellular energy, ceases and hypoxanthine (Hx) begins to generate. Therefore, the amount of Hx is an important indicator of fish freshness, and the nanomaterial-based sensors for Hx were summarized by Dervisevic et al. (2019). For determining Hx fish samples, Avan et al. (2023) designed an enzymatic determination method using a CUPRAC colorimetric sensor introduced by Bener et al. (2010). In this work, the Hx generated in stored fish sample was reacted with xanthine oxidase (XOD) to produce UA and H_2O_2 , which were both CUPRAC-reactive to produce a yellow color on the CUPRAC reagent-loaded Nafion membrane (Figure 7). It is known that the characteristic smell and taste in spoiled fish arise from relatively high levels of Hx, however sensory determinations allow to identify the beginning of spoilage and to determine the shelf life of fresh/canned fish.

4. Determination of Energetic Materials Using Different Analytical Techniques

Energetic materials (explosives, pyrotechnics, and propellants) are chemical substances or their mixtures that, upon release of stored chemical energy by an appropriate stimulus, produce a sudden gas expansion and thus exert high pressure (and thus destructive power) on its surroundings. They have been used for many different purposes from the first moment they were brought to

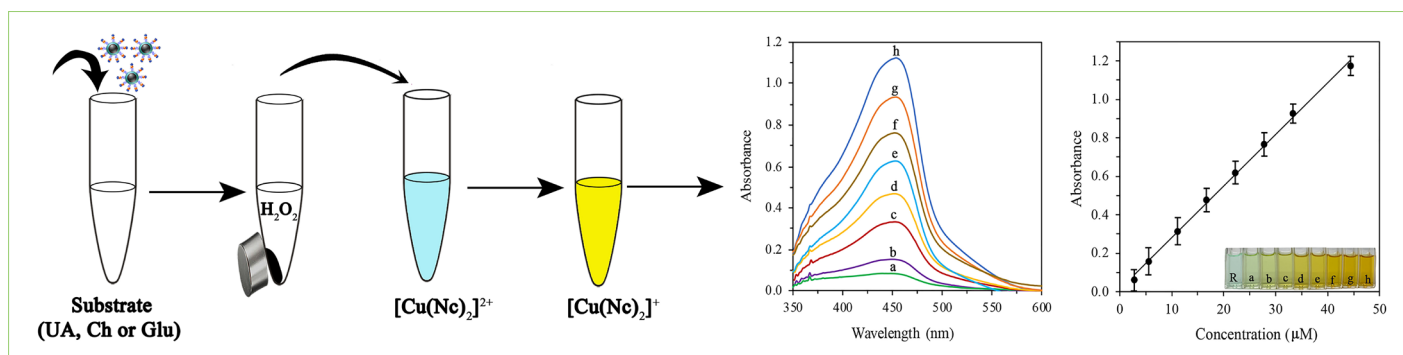


Figure 6. Determination of UA, Ch and Glu by using oxidase enzyme-immobilized MNPs nanobiosensor.

the stage of history by the Chinese in the IXth century until today (Agrawal, 2010), both positively (e.g., in construction, underground mining and surface hard rock quarrying) and negatively (e.g., in wars and terrorist attacks). Our research group has successfully designed novel methods of trace explosives detection and applied them to various energetic materials and their synthetic/real mixtures. Explosives can be basically divided into six classes according to their chemical structure: (1) nitroaromatics, (2) nitrate esters, (3) nitramines, (4) chlorates and perchlorates, (5) azides and (6) highly explosive materials (such as fulminates and organic peroxides) (Gaurav et al., 2007; Yinon, 1999). Of the various instrumental methods for explosive detection, we have specialized on spectroscopic and voltammetric methods, which are relatively simple, rapid, inexpensive, and on-site/in-field applicable.

4.1. Spectroscopic Determination of Energetic Materials

Color reactions allow selective qualitative analyses to be performed by the naked eye without any need for instrumentation (also suitable for cellular phone applications in the field), enabling their wide use for screening purposes. In addition to the methods designed by taking advantage of the chemical structure of energetic materials in color reactions, nanoparticle-based selective analysis has been successfully performed. The methods developed by our group for colorimetric testing of energetic materials are based on: (i) σ - and π - donor/acceptor interactions for nitroaromatics, (ii) determination of degradation products of nitramine and nitrate esters by Griess or Berthelot reactions (iii) analysis of H_2O_2 , the degradation product of TATP or HTMD, by redox-active colorimetric reagents, (iv) methods relying on miscellaneous methods incorporating nanomaterials.

Starting from 2004, the first of the colorimetric methods developed in our laboratories for explosives is for trinitrotoluene (TNT) and picric acid. The method is based on the formation of an ion pair between the Meisenheimer anion of TNT or picric acid in alkaline medium and cetyl pyridinium cation, followed by the extraction of the resulting uncharged structure into isobutyl methyl ketone (IBMK). The high water tolerance of the developed method compared to that of the literature method is very valuable for field analysis, especially in moist soils (Üzer et al., 2004). In the extractive-photometric method with superior properties developed later, the analysis of TNT solution in acetone-water was carried out by extraction with dicyclohexyl amine (DCHA)-IBMK organic solvent mixture. The pink color of the complex formed as a result of charge

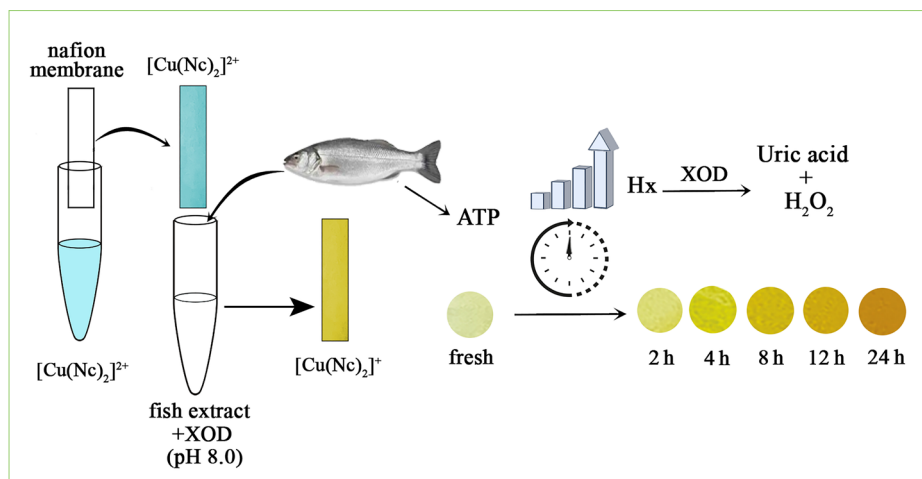


Figure 7. Determination of Hx extracted from fish sample let to stand at room temperature for different time periods using Cu(II)-Nc (CUPRAC reagent)-immobilized Nafion membrane.

transfer between TNT and DCHA was read at 531 nm. The method is not affected by water, various soil ions, and humic substances since both the extraction and color reaction basically takes place in the organic phase (Üzer et al., 2005). Additionally, the method is not sensitive to pH, because DCHA amine/ammonium base/conjugate acid pair with a low solubility in water constitutes a buffer system ensuring pH stability in extraction. Thus the method is extremely selective for TNT, not responding to other energetics and common interferents. For the determination of RDX, a nitramine class explosive, an alternative method based on the Berthelot reaction was developed. Berthelot reaction involves color development with phenol and hypochlorite in alkaline medium after hydrolysis of RDX with Zn dust to ammonia and low molecular weight amines (Üzer et al., 2008a). TNT, which interferes with the method, was removed by the extractive-photometric method previously developed by our group (Üzer et al., 2005). In addition, the interfering effect of ammonia and nitrate was eliminated by solubility difference and the method was successfully applied to explosives used for military purposes (Üzer et al., 2008a). Later analytical studies in our laboratory were carried out with sensors applied on solid surfaces. The first study for this purpose was the extraction of the Meisenheimer anion of TNT with the strongly basic Dowex 1-X8 anion exchange resin. Since the resin was in equilibrium with the solution, the concentration of TNT retained by the resin was determined by reading the orange color in the solution (Üzer et al., 2008b). A transparent sensor membrane was developed for the selective analysis of TNT using a polyvinyl chloride polymer matrix plasticized with dioctyl phthalate; DCHA was incorporated into this polymer membrane as a charge-transfer reagent and the resulting purple-red color *in situ*

was read at 530 nm (Erçağ et al., 2009). A new colorimetric method was developed for TATP and HMTD, which are peroxide-type explosives that are easily synthesized with readily available materials and widely used by terrorists. In addition, this method was adapted to a Nafion sensor membrane for the first time for peroxide-based explosives. Thus, a very important sensor suitable for use in the field for preventing/identifying terrorist actions has been developed (Eren et al., 2010). Especially considering costs per analysis, our group developed a very economical reflectometric method for both nitroaromatic and nitramine-class explosives. In the analyses performed on PVC tablets, reactions based on different colored Meisenheimer anions formation occurred for nitroaromatic class explosives, while pink color based on Griess reaction emerged if nitramine explosives were present. It can be presumed that this method will be suitable for analysing a large number of samples in contaminated/reclaimed land and criminal laboratories (Erçağ et al., 2011). Molecular spectroscopic methods were developed for the first time for HNS and NTO, which are non-sensitive explosives that have emerged as a requirement of modern armies. TNT shows an interfering effect during the analysis of both explosives. In simultaneous determinations, HNS and TNT, which both react with DCHA, are separated from each other by derivative spectroscopy. NTO and TNT are analyzed in basic medium. When NaOH is added to the medium, NTO forms a yellow Na^+NTO^- salt, while TNT gives a Meisenheimer anion extractable into IBMK by forming an ion pair with cetyl pyridinium bromide (Can et al., 2012). In parallel to the introduction of nanoparticles into our lives and their widespread use in different fields of science, nanoparticles have begun to be incorporated in the designed sensing systems due to their excellent surface

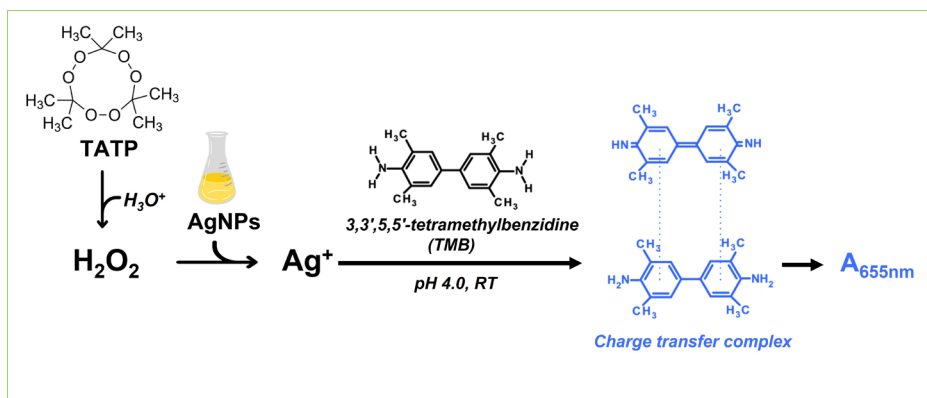


Figure 8. A schematic presentation for the detection mechanism of the developed H_2O_2 -selective colorimetric sensor for AgNP-based indirect determination of TATP after an acidic degradation.

properties. For the individual colorimetric identification of RDX and HMX in admixtures, which is a difficult task in view of their significantly similar chemical structure, the differences in their alkaline hydrolysis kinetics were exploited for the first time and a new gold nanoparticles (AuNPs)-based determination method was developed, where HMX required higher alkalinity at a higher temperature than RDX for degradation into nitrite. AuNPs were modified with 4-aminothiophenol (4-ATP), onto which RDX/HMX alkaline degradation product nitrite was linked to form a diazonium salt. Finally, by adding naphthyl ethylenediamine (NED) to the medium, a purple-colored azo dye was obtained through diazo-coupling (Üzer et al., 2014), with subsequent absorbance measurement. The developed AuNPs-based method was then adapted for PETN, a nitrate ester class explosive (Üzer et al., 2017b) and ammonium dinitramide (AND) used as rocket fuel (Mamatioğlu et al., 2021). The developed methods are the first nanoparticle-based assays for PETN and ADN. In addition, based on the developed method involving hydrolytic degradation to nitrite, a SERS method was introduced for the first time for RDX and HMX estimation (Güven et al., 2017). Nanoparticle-based methods have also been developed for TATP, usually occurring as a home-made explosive. H_2O_2 formed after acidic hydrolysis of TATP was degraded to hydroxyl radicals with Fe_3O_4 magnetite nanoparticles added to the medium at appropriate pH. These radicals formed led to the oxidation of the N, N-dimethyl-*p*-phenylenediamine (DMPD) probe to the pink-colored DMPD⁺ radical. At the same time, the sensitivity was increased by the retention of this radical cation on a Nafion membrane (Can et al., 2015).

(Üzer et al., 2017a) achieved an indirect determination of TATP based on partial oxidation of zero-valent AgNPs by H_2O_2 (released from acidic degradation of TATP)

to Ag^+ and then measurement of the absorbance of the blue-colored diimine (radical cation) of TMB at 655 nm, arising from TMB oxidation by Ag^+ (Figure 8). This practical method, which enables TATP to be detected by the naked eye after a simple preliminary hydrolysis process using strongly acidic cation exchanger resin (Amberlyst-15®), may alleviate the burden of criminology laboratories in terms of rapid screening and decision-making in crime scene investigations.

Two different types of titanium dioxide nanoparticles (TiO_2 NPs)-based H_2O_2 -selective colorimetric sensors were introduced and successfully used for TATP detection (after acidic hydrolysis) (Gökdere et al., 2019). The working principle of the first APTES-modified TiO_2 NPs (TiO_2 NPs@APTES) paper-like substrate was based on yellow-colored peroxy-titanate binary complex formation between Ti(IV) and H_2O_2 (the

latter released from TATP acidic hydrolysis), while that of the other on 4-(2-pyridylazo)-resorcinol modified- TiO_2 NPs (PAR@ TiO_2 NPs) solid sensor forming an orange-colored ternary complex among Ti(IV), PAR and H_2O_2 (the latter released from TATP acidic hydrolysis) (Figure 9). It is noteworthy that both nanosensors performed well, remaining stable for one week, thus providing an easy application for on-site analysis.

Highly sensitive colorimetric methods based on modified AuNPs and magnetite nanoparticles have been developed in our laboratories for TNT and tetryl explosives with similar nitroaromatic structures. A colorimetric sensor based on the aggregation of thioglycolic acid (TGA)- and diaminocyclohexane (DACH)-modified AuNPs (AuNPs@TGA/DACH) for the determination of TNT and tetryl at picomolar levels was developed (Ular et al., 2018). The sensing material was prepared by the ionic interaction between the $-\text{COO}^-$ group of TGA, which is attached to the AuNP surface *via* the $-\text{SH}$ group, and the (+)-charged ammonium-nitrogen of DACH (zwitterionic form at pH 8.2 as the arithmetic mean of pK_{a1} and pK_{a2} of DACH amino groups); this left one amine group of DACH free for interacting with the analyte (TNT). The detection mechanism of the sensor was based on the donor-acceptor interactions between the electron-rich free $-\text{NH}_2$ group of DACH and the electron-deficient $-\text{NO}_2$ groups of TNT/tetryl, causing aggregation of AuNPs@TGA/DACH (Figure 10). The sensitivity of the sensor was at picomolar level, potentially enabling the precise detection of trace amounts of TNT and/or tetryl in post-blast residues.

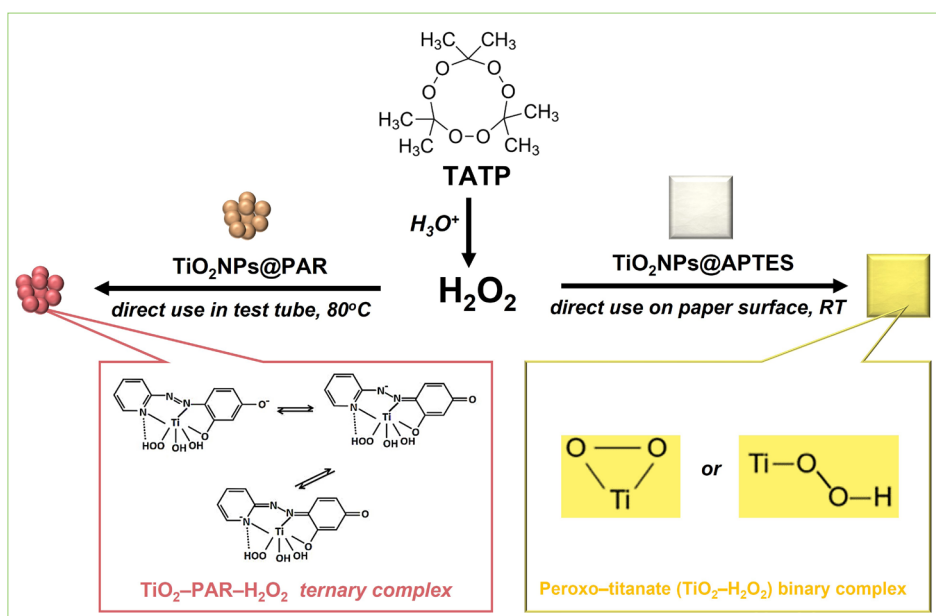


Figure 9. Schematic presentations of the proposed mechanisms for TATP detection using both TiO_2 NPs@APTES paper-based and TiO_2 NPs@PAR sensors.

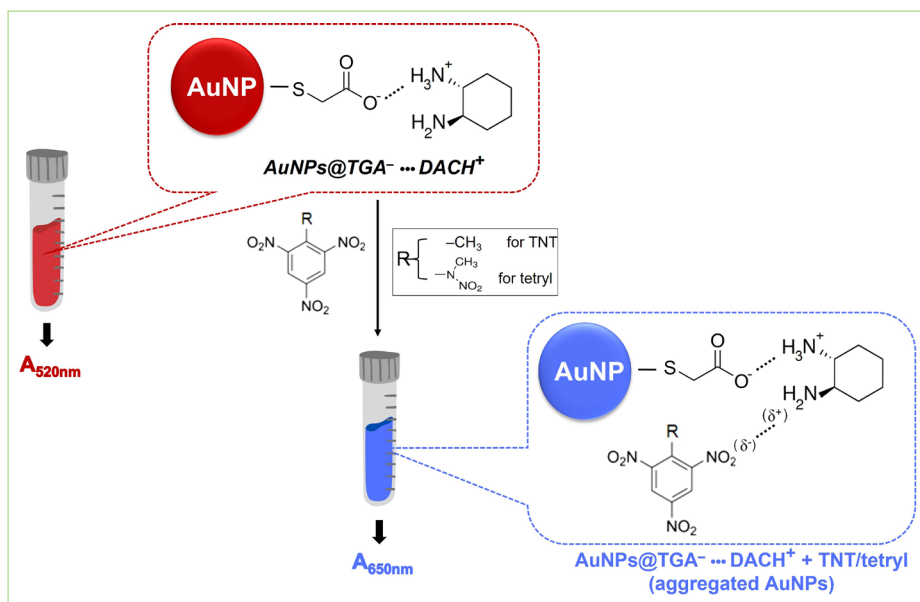


Figure 10. Schematic presentation of the proposed mechanism for TNT/tetryl detection using TGA- and DACH-modified AuNPs.

A simple and low-cost colorimetric sensor for nitroaromatic compounds (TNT and tetryl) was designed by (Özcan et al., 2019) using cetyltrimethylammonium bromide (CTAB)-stabilized AuNPs modified with diethyldithiocarbamate (DDTC), the parent compound of thiocarbamate pesticides. The detection principle of the sensor was based on Meisenheimer complex formation as a result of donor-acceptor interactions between the partial positively charged amine-N of DDTC (of which the electronegativity of thiocarbonyls was attenuated after binding to the Au surface through the S-atom, thereby increasing the basicity of amine-N) and partially negatively charged $-\text{NO}_2$ groups of TNT/tetryl at near neutral pH (Figure 11). The fact that the formation of the Meisenheimer complex, which is usually carried out under alkaline conditions, could be accomplished at neutral pH with an inexpensive ligand makes the sensor remarkable.

Ethylenediamine (EDA)-modified MNPs were synthesized with the polyol technique in one pot reaction (Yardımcı et al., 2021). This is the first study involving the design of a unique reusable, selective, low cost diamine-modified MNPs-based colorimetric sensor using the principle of charge-transfer complex formation for colorimetric detection of TNT/tetryl *via* two different methods combined in the same sensor. While the first of these methods is based on the direct (donor-acceptor) interaction of TNT and tetryl with EDA-modified MNPs (method-1), the other method is based on the nanozyme ability of EDA-modified MNPs to catalyze H_2O_2 oxidation to produce a blue-colored charge transfer complex from oxidized TMB, where TNT and tetryl in the medium caused bleaching of this color by

inhibiting the catalytic ability of nanoparticles through charge-transfer interaction (method-2) (Figure 12).

Another gold nanoparticle-based colorimetric probe (AuNPs@Thr, for determination of TNT and tetryl) modified with thiram, a cheap insecticide of wide use, was developed (Demircioğlu et al., 2022). Upon interaction of the probe with TNT (or tetryl), an aggregation-based bathochromic shift was observed in the characteristic surface plasmon resonance (SPR) absorption wavelength of AuNPs. In addition, this shift took place at room temperature within a very short time with high sensitivity (Figure 13).

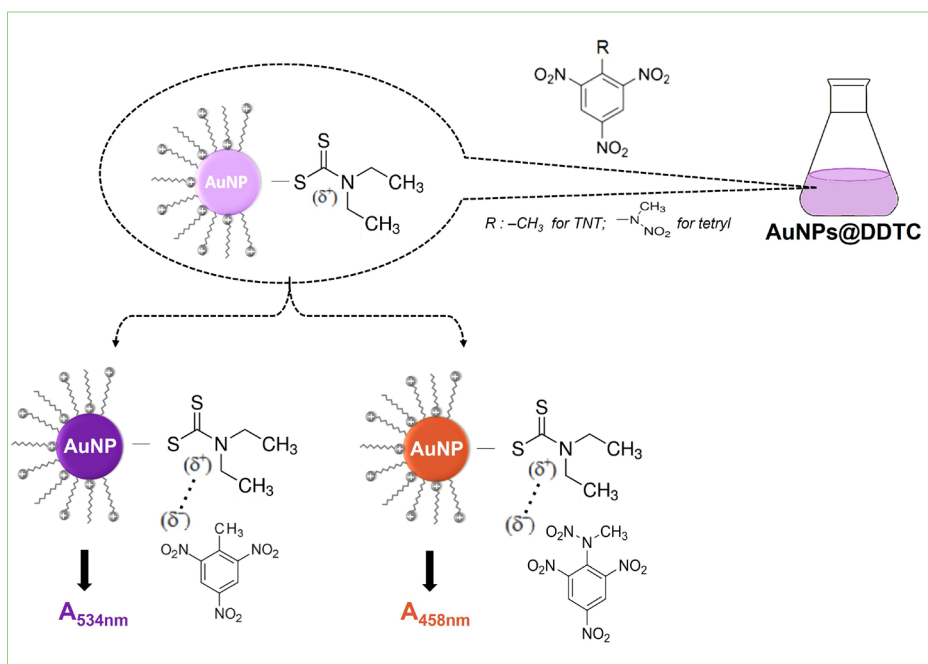


Figure 11. Schematic presentation of the proposed mechanism for TNT/tetryl detection using DDTC-modified AuNPs.

Şen et al. (2021) developed a simple method for TNT estimation based on the fluorescence quenching of rhodamine 110 dye with FRET mechanism, owing to the primary amine groups of rhodamine 110 (different from other rhodamines) capable of donor-acceptor interaction with TNT. Bener et al. (2022b) designed protamine-capped gold nanocluster (PRT-AuNCs) sensor for TNT, in which the observed turn-on fluorescence arose from aggregation-induced emission enhancement; protamine is a highly positively-charged polypeptide primarily composed of arginine. As a result of the donor-acceptor interaction between the non-fluorescent Meisenheimer anion formed from TNT and the amino groups of weakly fluorescent protamine, the PRT-AuNCs aggregated, accompanying an enhanced fluorescence intensity with a large Stokes shift, forming the essence of this sensitive TNT assay. Şen et al. (2022a) brought a different perspective to TNT determination without using any nanoparticles; the ligand for TNT was cysteamine (CysN) in a deep eutectic solvent (DES) composed of choline chloride and ethylene glycol. The method did not require additional alkalinity as that in water for stabilizing the Meisenheimer anion derived from TNT; CysN-TNT donor-acceptor charge transfer complex was stable in DES unlike in water. On the other hand, two modified gold nanoparticle-based colorimetric methods were developed for ammonium perchlorate (AP), used as an oxidizer in rocket systems with its high oxygen content. The first sensor is based on attaching methylene blue (MB), a cationic dye with a planar structure, onto a negatively-charged AuNP surface by electrostatic interaction, and the

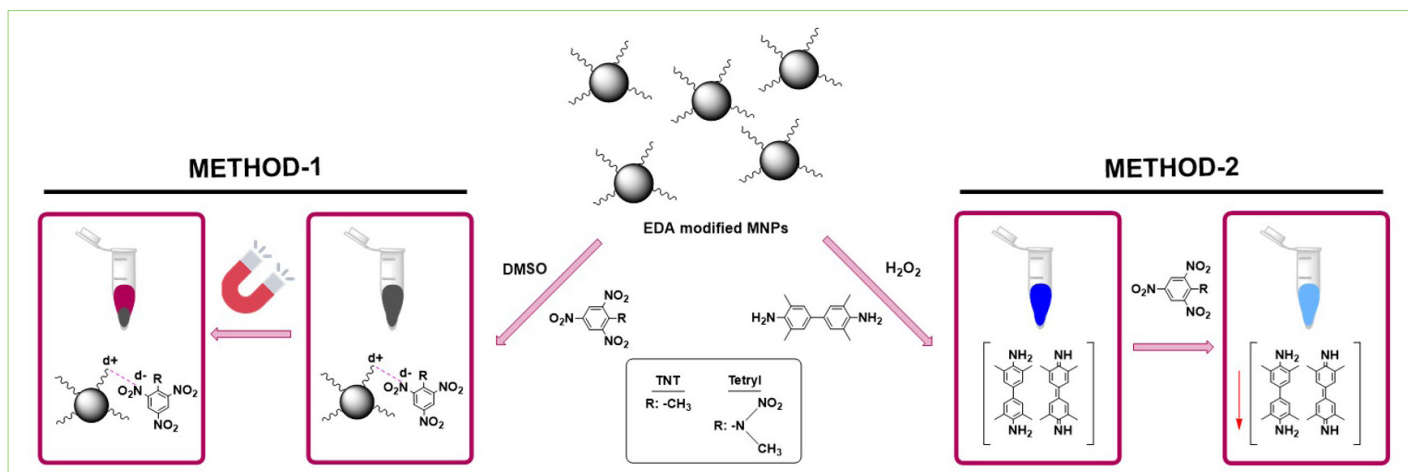


Figure 12. Schematic presentation of the proposed mechanism for TNT/tetryl detection using EDA-modified MNPs.

dye-forming an ion pair with the large oxyanion perchlorate. Since the formation of the ion pair reduced the surface charge of AuNPs, partial aggregation occurred, causing a red shift in the surface plasmon resonance (SPR) absorption band of AuNPs. This work is the first colorimetric sensor developed to detect propellant AP in the presence of other explosives and interferences, exploiting the extraordinary physico-chemical properties of gold nanoparticles (Keskin et al., 2020) (Figure 14).

In the other spectroscopic sensor for AP, AuNPs were modified with imidazolium-based ionic liquid in the cationic and zwitterionic form (Keskin et al., 2022). The ionic liquid was attached to the AuNPs

surface by electrostatic interaction and in an alkaline medium, the zwitterionic ionic liquid is in reverse micelle form. The less hydrated perchlorate ion diffused into these reverse micelles, bringing together opposite charges of the zwitterionic ionic liquid bound to the IL@AuNPs, leading to charge neutralization of nanoparticles. When the perchlorate anions inter-bridge the IL@AuNPs, partial aggregation occurred, enabling the detection of ClO_4^- . This work is a pioneering study in which the overall negative charge of IL@AuNPs was exceptionally decreased by the negatively charged perchlorate anion and the nanoparticles were aggregated by anion- π interaction of perchlorate anion with the zwitterionic form of IL@AuNPs so

as to bring opposite charges face-to-face (Figure 15).

Our group has also developed novel methods for quantifying insensitive explosives (*i.e.* munitions of reduced vulnerability to heat and shock) like 3-nitro-1,2,4-triazole-5-one (NTO) and nitroguanidine (NQ). In a method developed for NTO, the L-cysteine functionalized gold nanoparticles (Cys-AuNPs) had an ammonium group that attracted the NTO anion electrostatically. In the presence of cupric ions, this led to the creation of a Cu^{2+} -coordination complex among Cys-AuNPs, followed by their aggregation and subsequent color change from red to violet (*i.e.* SPR band maximum shifted from 520 to 650 nm). Thus, this ratiometric sensor determined the analyte by correlating the absorbance ratio ($A_{650\text{nm}}/A_{520\text{nm}}$) to NTO concentration (Türkekul et al., 2019). Durmazel et al. (2022) demonstrated a new detection mechanism for quantifying NTO by exploiting the anti-aggregation behavior of AuNPs (as an unlabeled nanosensor) based on the strongly competitive and supramolecular interactions of the target analyte (NTO) with melamine (MEL)-functionalized gold nanoparticles (MEL-AuNPs) via primarily hydrogen bonding. In this respect, MEL-induced aggregation of AuNPs was delayed/inhibited in the presence of increasing concentrations of NTO leading to color change (from purplish blue to ruby red color) that can be observed with the naked eye even at fM levels (Figure 16). The developed sensor had remarkable performance in terms of its NTO detection capability at sub-fM levels with the use of label-free AuNPs measurable with an inexpensive colorimeter; the sensor displayed high recoveries for NTO from synthetically contaminated (NTO-adsorbed) soil samples and from complex mixtures of similar substances through a simple acidic extraction process. It also showed the boundaries of the capabilities of noble metal

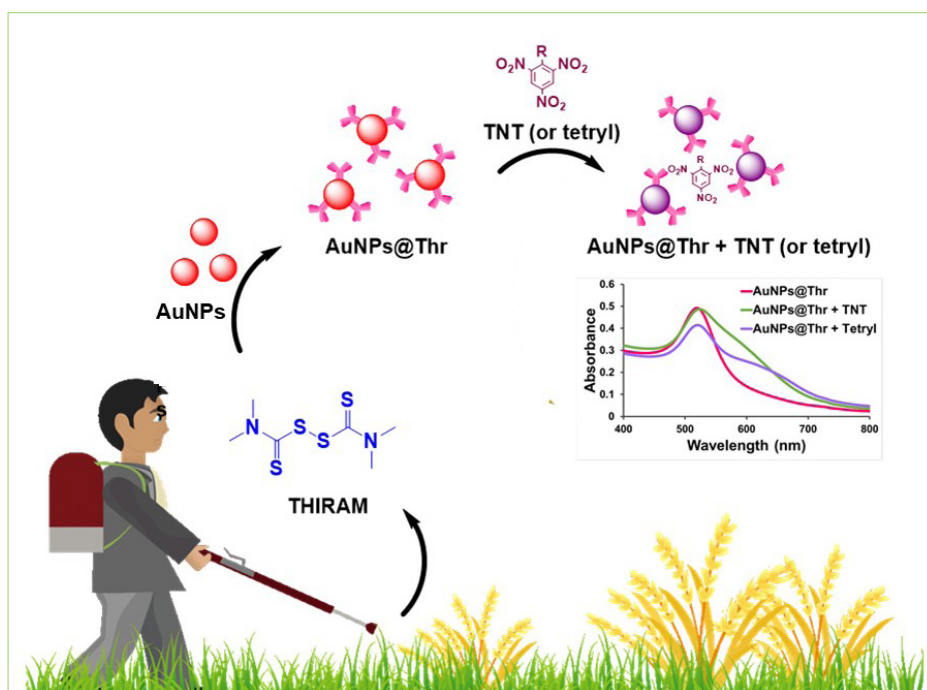


Figure 13. Schematic presentation of the proposed mechanism for TNT/tetryl detection using AuNPs@Thr probe.

nanoparticles in the detection of femto-molar level analytes merely using color changes from aggregation/disaggregation reactions.

For the determination of nitroguanidine (NQ), (Can et al., 2023) developed a colorimetric strategy based on the aggregation of uric acid-modified gold nanoparticles (AuNPs@UA) with NQ. In this study, UA was readily bound to the surface of AuNPs via the imide group, and the sensing principle was based on the hydrogen-bonding interaction between the $-NH$ (H-donor and/or acceptor) group of UA and the $-NO_2$ (H-acceptor), the $-N$ (H-acceptor) and the $-NH_2$ (H-donor and/or acceptor) groups of NQ, which led to aggregation of AuNPs (Figure 17) causing the red color of AuNPs to shift to bluish purple. The method was not affected by many common interferents; this selectivity possibly arose from the special hydrogen bonding interaction of NQ inter-bridging AuNPs@UA, as the NQ molecule, having both charge donating amine and electron-withdrawing nitro groups in its structure and being chargeless over a wide pH range, can neither form charge-transfer nor electrostatic interactions with UA-functionalized AuNPs.

For TNT and picric acid, we introduced to world literature carbon quantum dots-based fluorometric methods which have an increasing trend for sensor applications and allow selective analysis. The nitrogen-doped carbon quantum dot (N-CQDs) were synthesized via the reflux method by choosing citric acid as carbon source and ethylene-diamine together with diamino cyclohexane as nitrogen sources, without a need for

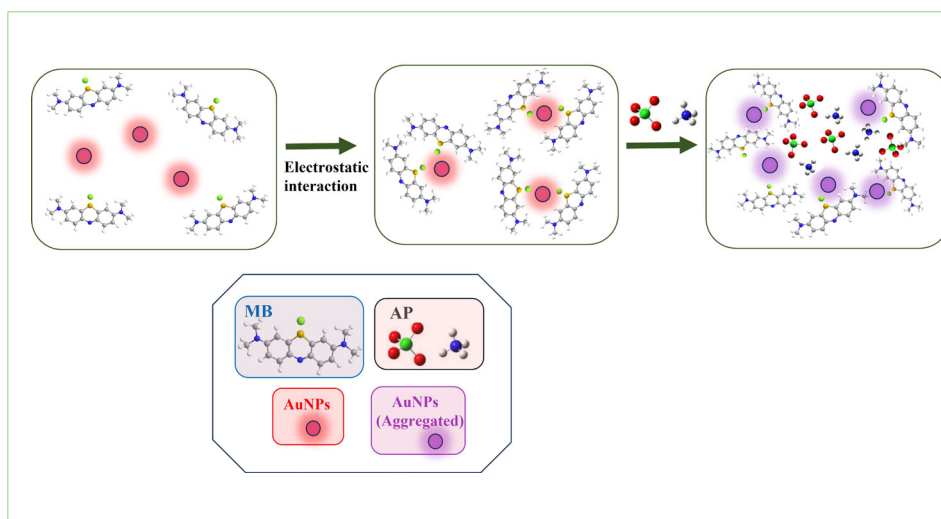


Figure 14. Mechanism describing the aggregation of MB@AuNPs by charge neutralization for determination of ammonium perchlorate.

organic solvents (Koç et al., 2022). N-CQDs, which have high photostability and show blue emission, trap nitrogen atoms on their surface and show high sensitivity only to TNT among different types of explosives (*i.e.* belonging to nitroaromatic, nitramine, and nitrate ester categories), without a need for additional chemical modification (Figure 18). Another fluorescent polyethyleneimine-capped carbon quantum dots (PEI-C-dots) probe was synthesized by a microwave-assisted technique using polyethyleneimine (PEI) and citric acid precursors, which was used to detect TNT. The sensing mechanism of TNT is based on fluorescence quenching as a result of the donor-acceptor interaction between the Meisenheimer anion of TNT

and PEI on the PEI-C-dots surface (Şen et al., 2022b).

A CQDs-based method was devised for the fluorometric determination of picric acid (PA), curcumin (CUR), and amine-type antioxidants (namely N-phenyl-p-phenylenediamine, N-phenyl-1-naphthylamine, N-phenyl-2-naphthylamine, and diphenylamine), the latter used as propellant preservatives. CQDs were synthesized by reflux method in water-soluble form using the molecular precursors of citric acid and N-(4-methylpyridin-2-yl)ethane-1,2-diamine (Benli et al., 2023) (Figure 19). Electrostatic and H-bonded complexes enabled the detection of PA and CUR, respectively, where PA could be recognized at

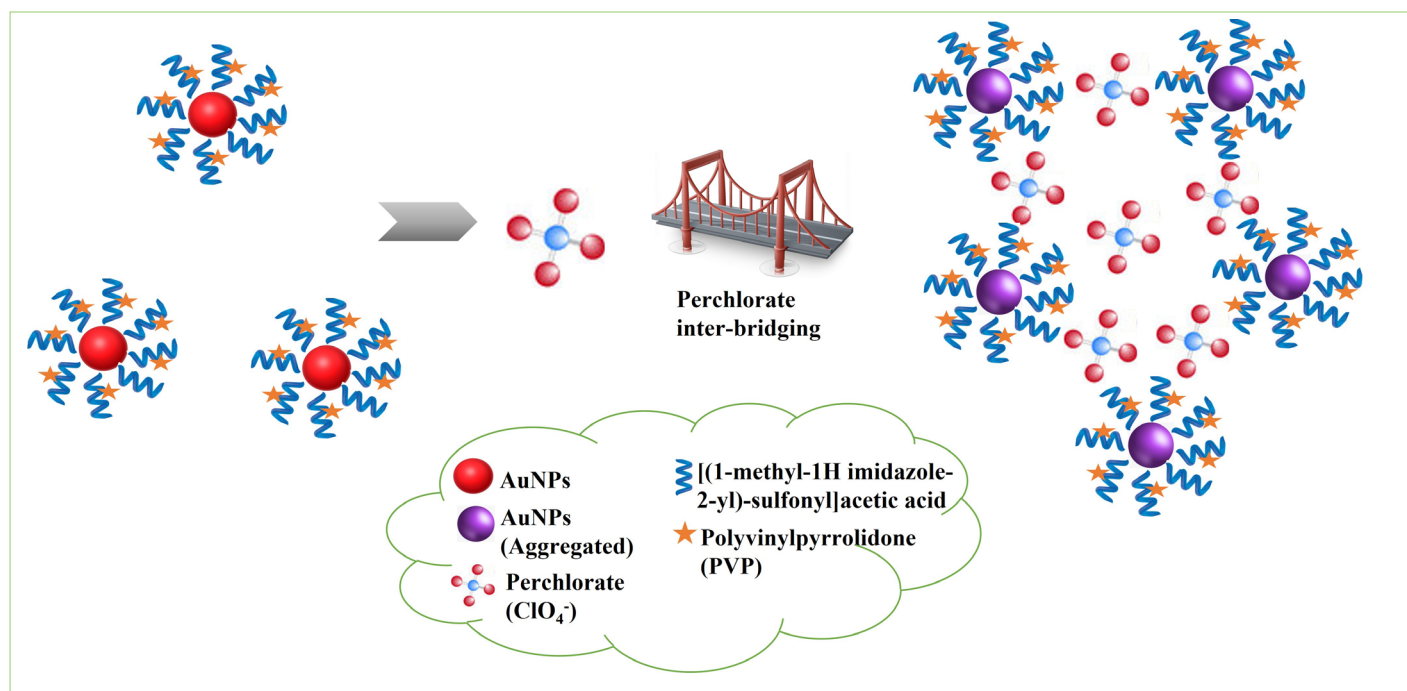


Figure 15. Aggregation of IL@AuNPs with perchlorate, forming the basis of ClO_4^- sensing.

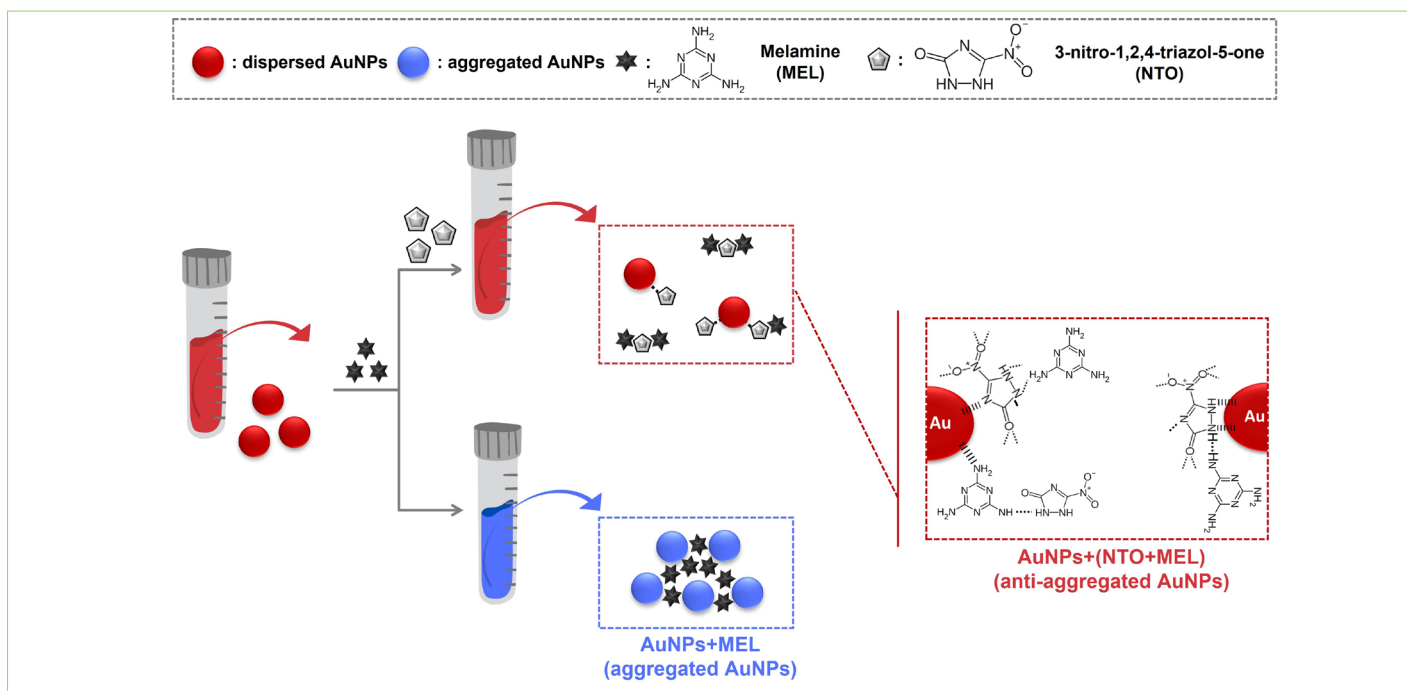


Figure 16. Schematic presentation of the mechanism of NTO detection using unlabeled AuNPs acting as a disaggregation sensor in the presence of MEL.

sub-nanomolar concentrations by static fluorescence quenching of its CQD-complex.

4.2. Electrochemical Determination of Energetic Materials

Spectroscopic and chromatographic methods are sometimes criticised for being rather laborious and expensive, especially in combined use, as in HPLC/MS applications. Instead, because of their straightforward operation, low cost, high sensitivity, and selectivity, quick response, portability, and ability to distinguish between electroactive species, electrochemical (EC) technologies provide several advantages for explosives detection (Arman et al., 2022).

Electrochemical analysis of energetic compounds can be carried out using solid working electrodes (glassy carbon, platinum, or gold), carbon-fiber (CF) electrodes, screen-printed electrodes (SPEs), and mercury-based electrodes (the latter having a decline because of toxicity problems). Besides, sensor working electrodes can be developed for sensitive and selective electrochemical determination of energetic substances by modifying the surfaces of these electrodes with conductive polymers such as polyaniline (PANI), poly-*o*-phenylenediamine (P-*o*-PDA) and polycarbazole (PCz), decorated with nanomaterials such as nanoparticles (NPs), carbon nanotubes (CNTs), and graphene (GR) to give rise to enormous surface areas, improved surface activity, and distinctive electrical and optical features (Sağlam et al., 2018). Moreover, molecular imprinting/memory techniques can be used to create sensing materials with cavities that

can identify a specific molecule/ion in terms of its size, geometry, functional groups and physico-chemical form. High selectivity and affinity for the target molecule are the main benefits of molecularly imprinted polymers (MIPs) (Spéjel et al., 2002).

The most prevalent reaction mechanisms for explosives analysis employing the functionalized electrodes are σ - π / π - π interactions, charge-transfer and electrostatic complexations, and hydrogen bonding. Additionally, nanomaterials can function as electrochemical reaction catalysts, which lowers the activation energy barrier for the oxidation/reduction reaction of energetic materials as target analytes. Moreover, a considerably more accurate and sensitive estimation of explosive compounds is possible by combining the sensors made of conductive

polymers with nanoparticles. In particular, the selectivity and sensitivity of explosive detection are dramatically improved when MIP sensors are coupled to these materials. In addition, MIP technology offers the explosive detection sector a variety of items and compact solutions.

Our group has prepared various sensor working electrodes and detection methods for the electrochemical determination of energetic substances based on the aforementioned mechanisms. Firstly, we reported a cyclic voltammetric (CV) method for analyzing nitroaromatic (trinitrotoluene (TNT) and dinitrotoluene (DNT)) and nitramine type energetic materials (hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)) in synthetic and real mixtures

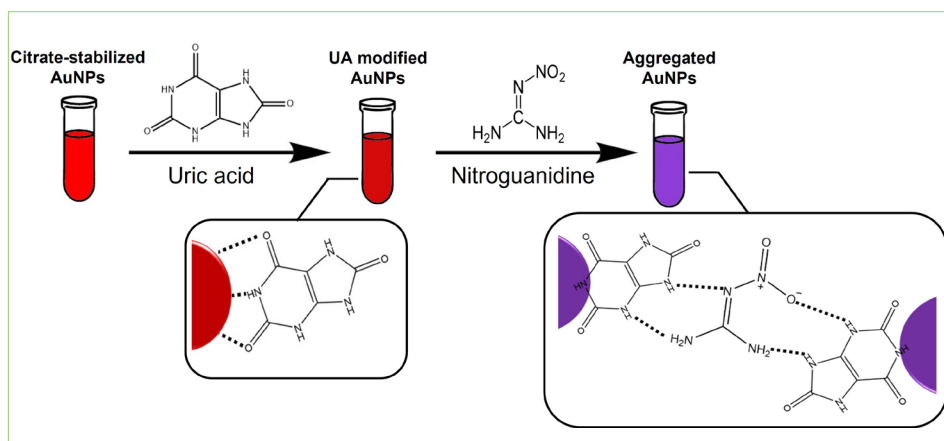


Figure 17. Schematic representation of the colorimetric strategy for NQ sensing via hydrogen bonding-induced aggregation of AuNPs@UA.

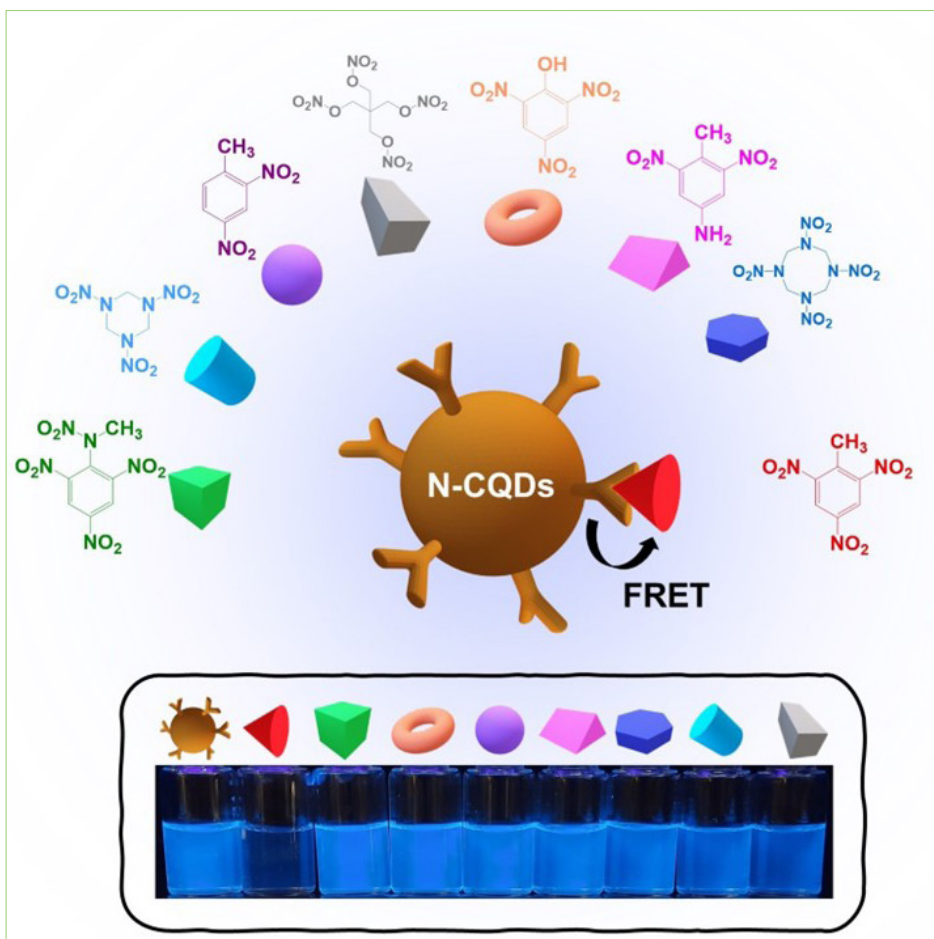


Figure 18. Schematic representation of TNT detection with N-CQDs.

using a glassy carbon electrode in acetonitrile (AcCN) medium. These materials can be determined easily because they contain aromatic -NO_2 or cyclic 4N- NO_2 groups, which can be reduced at suitable potentials. The limit of detection (LOD) was between 10.2-11.7 mg L^{-1} for these analytes (Üzer et al., 2013).

We developed an electrochemical sensor electrode for nitroaromatic explosives analysis (TNT, DNT, Tetryl) based on the electropolymerization of *o*-phenylenediamine (*o*-PDA) and aniline (ANI) monomers on the GC electrode surface using the CV method, and gold nanoparticles (AuNPs) were electrochemically deposited on the formed electrode. Through their donor/acceptor interactions with the *o*-PDA-ANI electropolymers on the modified electrode, nitroaromatic compounds were identified/quantified. The improved sensitivity was attained by π - π and charge-transfer (CT) interactions between the electron-deficient nitroaromatic compounds and σ - π -donor (electron-rich) amine/aniline groups attached to AuNPs. Moreover, Comp B, Octol, and Tetrytol, used as nitro-explosive mixtures for military purposes, were effectively analyzed using the suggested methodologies (Sağlam

et al., 2015). In another work devised for the electrochemical detection of nitroaromatic (TNT and DNT) and nitramine type (RDX and HMX) energetic materials, we proposed molecularly imprinted sensor electrodes relying on the electrochemical modification of GC-electrode with nitro-energetic memory-*poly*(carbazole-aniline) copolymer using the CV method, with subsequent decoration with AuNPs. The nitro-energetic materials were separately analyzed using these MIP sensor electrodes in various mixtures. The

limit of detection (LOD) was between 10-30 $\mu\text{g L}^{-1}$ for the analytes. The main mechanism of detection was π - π interaction between the molecules, and these MIP electrodes enhanced the sensitivity for nitro-energetic materials by decreasing the LODs almost 100-fold compared to those found with non-imprinted analogic electrodes. Additionally, the MIP electrodes were highly selective in the presence of synthetic explosive mixtures, military explosives, common soil ions, and potential interferents such as electroactive camouflage materials, displaying repeated use without loss of activity for 15 days (Sağlam et al., 2018). Furthermore, sensitive and selective determination of TNT, DNT, 2,4-dinitrophenol (2,4-DNP), tetryl, and picric acid (PA) was achieved using glassy carbon/multi-walled carbon nanotubes/polyethyleneimine (GC/MWCNTs/PEI) electrode coated with gold nanoparticles (AuNPs) *via* differential pulse voltammetry (DPV) (Figure 20). Increased analyte affinity toward the modified GCE was made possible *via* π - π and charge-transfer (CT) interactions between the electron-deficient nitroaromatic explosives and electron-rich amine groups of PEI that were coupled to gold nanoparticles. The limit of detection (LOD) was between 15-135 $\mu\text{g L}^{-1}$ for analytes. For analysis in synthetic explosive combinations and military composite explosives (Comp B and Octol), the suggested method was successfully used; common soil ions and potential interferents such as electroactive camouflage materials did not adversely affect this method (Arman et al., 2022).

We also performed studies where the main mode of modified electrode-analyte interaction is hydrogen bonding. The accurate and selective detection of peroxide-based explosives (PBEs) in-field/on-site is a major analytical problem because most methods claiming to identify PBEs are indirect and actually detect H_2O_2 , emerging as the by-product of their acidic hydrolysis. Using a well-dispersed multiwalled carbon

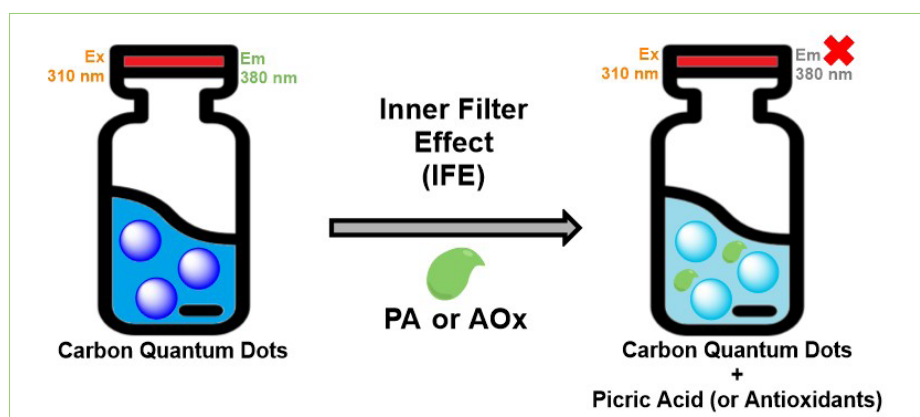


Figure 19. Schematic representation of PA detection with CQDs *via* static quenching of fluorescence.

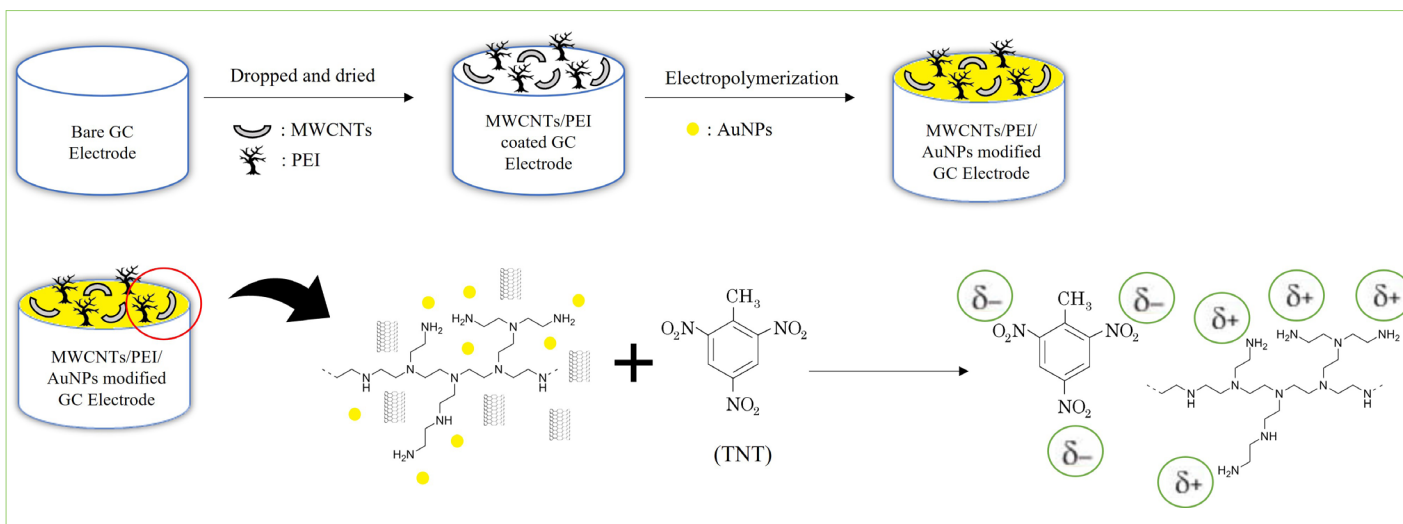


Figure 20. Schematic display of the GC/MWCNTs/PEI electrode preparation and reaction mechanism for nitro-explosive detection (Arman et al., 2022).

nanotubes/polyethyleneimine (MWCNTs/PEI) modified GC electrode, we described an electrochemical sensor for the direct detection of triacetone triperoxide (TATP) and hexamethylenetriperoxide diamine (HMTD) via DPV method. Our sensor electrode responded to intact TATP solutions in neutral medium, which is a clear benefit over previous comparable methods, because TATP was not converted to H_2O_2 with acids/bases prior to electrochemical analysis. The interaction of polyelectrolytes (such as

polyethyleneimine) with TATP molecules, *i.e.* H-bond formation of H-atoms in the N-H groups of PEI with the O-atoms in the peroxide linkage C-O-O-C of TATP, enabled to reach low detection limits; 1.5 mg L^{-1} for TATP and 3.0 mg L^{-1} for HMTD. This approach was not affected by common ions, other explosives, and electroactive substances carried by passengers (such as detergent, sugar, sweetener, and paracetamol-caffeine-based analgesics) (Arman et al., 2021). In another work, by

electrochemical modification of GCE surface with PBEs-memory polycarbazole (PCz) film decorated with AuNPs, we created a novel electrochemical sensor for the direct sensitive/selective detection of PBEs (TATP and HMTD) via DPV method (Figure 21). The essential mechanism of PBE detection of this polycarbazole-modified MIP-sensor electrode was hydrogen bonding, having LOD values around $15 \mu\text{g L}^{-1}$. In the presence of nitramine, nitrate ester, and nitroaromatic energetic substances, the sensor

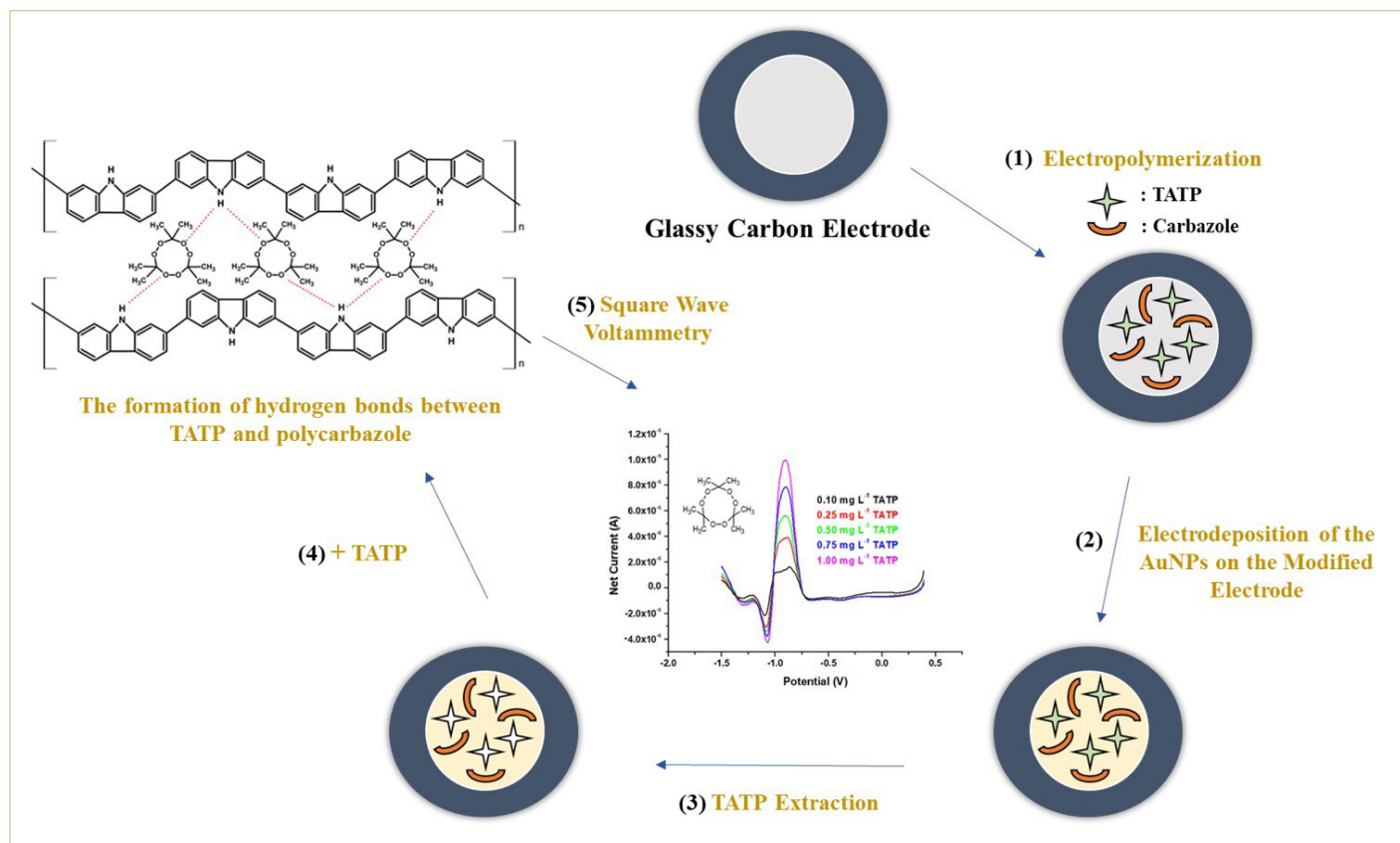


Figure 21. Schematic presentation of polycarbazole-modified MIP-sensor electrode preparation and reaction mechanism for PBEs detection (Sağlam et al., 2022).

electrodes were able to individually quantify intact TATP and HMTD. This was the first MIP electrode for PBEs to achieve such low LODs, and contaminated clay soil samples were used to statistically validate the DPV method (Saçlam et al., 2022). For selective electrochemical determination of the insensitive energetic, nitroguanidine (NG), the GC electrode was modified with well-dispersed multi-walled carbon nanotubes (MWCNTs)

and polyvinylpyrrolidone (PVP) mixture using the square-wave voltammetry (SWV) method. The intermolecular hydrogen bonding between the carbonyl group of PVP on the prepared electrode and the three hydrogen atoms of NG enabled to quantify NG with a low LOD of 0.6 mg L⁻¹, and both synthetic and real energetic material mixtures did not interfere with this procedure. The prepared electrode could be used for one week

without any cleaning (Alassane Moussa et al., 2022).

As a result, electrochemical methods constitute a highly good substitute for chromatographic and spectroscopic methods due to their low cost, low chemical consumption, good selectivity and sensitivity, precision, easy applicability and portability, particularly enabling field analysis.

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