Implementation of nanotechnology in drug analysis as a new solution for old problems: a review

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There is an increasing interest in nanoparticles nowadays. In this review, we will enlighten the use of nanoparticles for detection and quantification of some drugs, antibiotics, antivirals, and amino acids using different analytical methods.

Keywords:
analytical methods, drugs, nanoparticles, preparation

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L-cysteine analysis

Nanoparticles (NPs) were used for quantification of L-cysteine using multiwalled carbon nanotubes (MWCNTs) [1,2] or silver nanoparticles (AgNPs) [3] before measuring by ultraviolet (UV)–visible (Vis) spectrophotometer. Preparation methods of such NPs in the literature were found to follow a specific scheme as follows.

The pretreatment of MWCNTs occurred in two steps: first, oxidation by acids was done by refluxing 500 mg of MWCNTs in 50 ml H_2SO_4-HNO_3 solution (75 : 25, V/V) for 4 h at a temperature of 60°C. The CNTs were filtered and washed with deionized water, and then dried under vacuum at 100°C for 5 h. After that, the dried MWCNTs were immersed in 1 ml dimethyl formamide, then 10 ml of thionyl chloride was added, and the mixture was stirred for 1 h, and then it was kept for 1 day under nitrogen at 80°C. After that, the MWCNTs were washed with acetone and allowed to dry under vacuum for 1 day at 25°C [2]. Overall, 500 mg of pretreated MWCNTs was suspended in acetonitrile (20 ml) for 10 min. Then 1 ml of methyl acrylic acid and 50 mg of $NaHCO_3$ were added, and the whole mix was allowed to stand under nitrogen for 2 h at 80°C. After that, the resulting methyl acrylic acid (MAA)/MWCNTs were filtered and washed with deionized water and dried under vacuum [2]. In a 100-ml round-bottom flask, 20 ml of acetonitrile was added, and then 1 mmol of L-cysteine (purchased from Sigma-Aldrich (St Louis, Missouri, USA; http://www.sigma-aldrich.com) and 300 mg of MAA/MWCNTs along with 4.0 mmol of MAA were added to the flask, and the whole mixture was incubated at 25°C for 60 min. After that, 20 mmol of ethylene glycol dimethacrylate was added, and the mixture was washed with nitrogen for the purpose of oxygen removal. The washing process lasted for 10 min. Then the polymerization process started by adding 30 mg of azobisisobutyronitrile and allowing it to react with the mixture for 24 h at 60°C. A solution of 10% acetic acid in ethanol was used to wash the resulting polymer for the removal of template. The washing was considered complete when the template was no longer detected by the spectrophotometer. Then the resulting molecularly imprinted polymers MWCNTs were dried under vacuum for 0.5 h at 60°C [2]. An aliquot of a 50 ng/ml L-cysteine solution was added to a 250-ml beaker, and the pH was brought down to 6.5 using HCl. After that, 50 mg of the previously prepared molecularly imprinted polymers/MWCNTs was added to the mixture, which was then agitated for 20 min. Then the solution was centrifuged for 3 min at 3500 rpm to remove the supernatant, and the adsorbents were

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eluted using 1.5 ml of 10% acetic acid in methanol. Then, 75 μl of 0.0005 molar DDO was added, and the solution was allowed to stand for 20 min at room temperature. After that, the solution was measured spectrophotometrically at 478 nm using a UV–vis spectrophotometer model SPECORD 210 (Analytik jena, Germany) with a 300-μl quartz microcell (Fisher Co., Baden-Württemberg, Germany) [2]. Another method for determination of L-cysteine employed AgNPs as follows: a solution of HNO_3/HCl was prepared, and then directly used to soak all glassware to be used in this method. After that, the glassware was washed with water and dried. A 7.0×10^{-5} M solution of $AgNO_3$ was prepared, and an appropriate amount of polyvinylpyrrolidone was added. Then 10 ml of 3×10^{-2} molar sodium borohydride solution was added drop wise with vigorous shaking. When the colorless solution turned into light yellow, the shaking was stopped and the formed AgNP solution was stored in dark place at 25°C for 24 h, to avoid the possible interference from the hydrogen gas that results from the interaction between the sodium borohydride and water [3].

Dexmedetomidine analysis

Sodium dodecyl sulfate (SDS)-modified maghemite nanoparticles (MNPs) have been developed for removal, preconcentration, and spectrophotometric determination of trace amounts of a naphthalene analog of anesthetic dexmedetomidine at 280 nm using a double-beam UV–vis spectrophotometer (Perkin Elmer, Akron, Ohio, USA, Lambda 45) [4]. The used 4-(1-(naphthalene-1-yl) ethyl)-1H-imidazole (NMED) was synthesized according to a previously reported method. After preparation of MNPs [4], they were coated with SDS using 1.0 ml of 5% SDS solution to each 0.1 g of the MNPs, followed by vigorous stirring for 1 min. After that the beaker was put on a magnetic plate and allowed to stand until the MNPs settled at the bottom of the beaker. Then, the supernatant was decanted, and the MNPs were washed with double-distilled water to remove the excess SDS [4]. Overall, 150 ml of a solution of 10^{-4}–10^{-6} M of NMED was prepared and added to 0.07 g of SDSMNPs, and the pH of the solutions was adjusted to 3.0 and then the solutions were stirred for 0.5 h. A gradual decrease in NMED concentration was caused by its adsorption on the surfacy of the SDSMNPs. After that, the MNPs loaded with NMED were isolated from solution using a magnetic plate, and the NMED was separated from the MNPs using 1.0 ml of 50% acetonitrile in ethanol, and the concentration of NMED was determined by a spectrophotometer at 280 nm [4]. The efficiency of the MNPs to remove the NMED was calculated as follows:

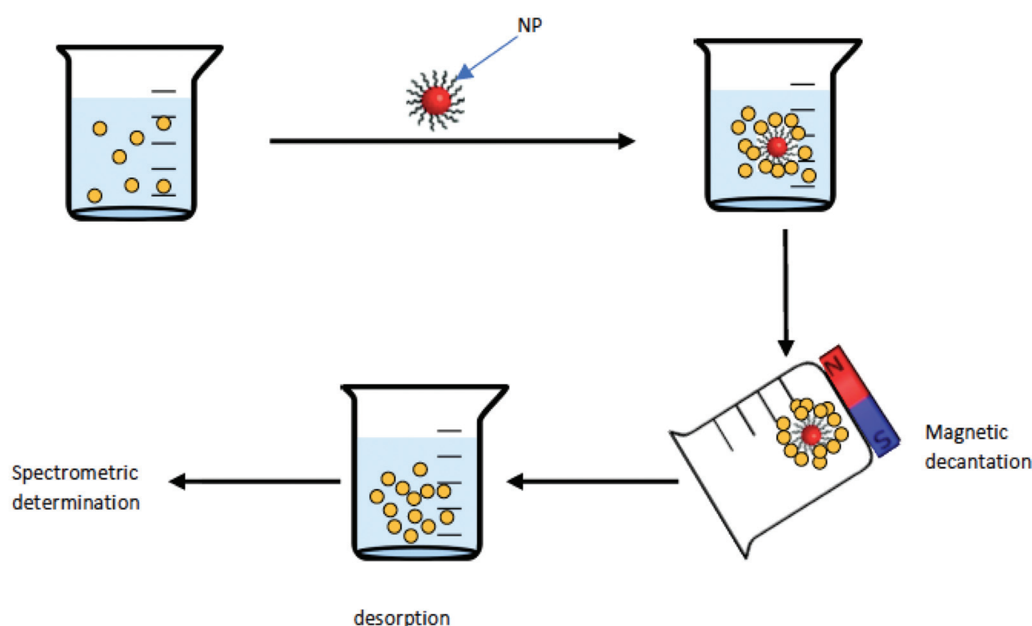
$$\%\mathrm{Re} = [(C_0 - C_t)/C_0] \times 100.$$

where C_0 and Ct are the initial and final (after adsorption) concentrations of the drug in mol/l [4] (Fig. 1).

Clozapine analysis

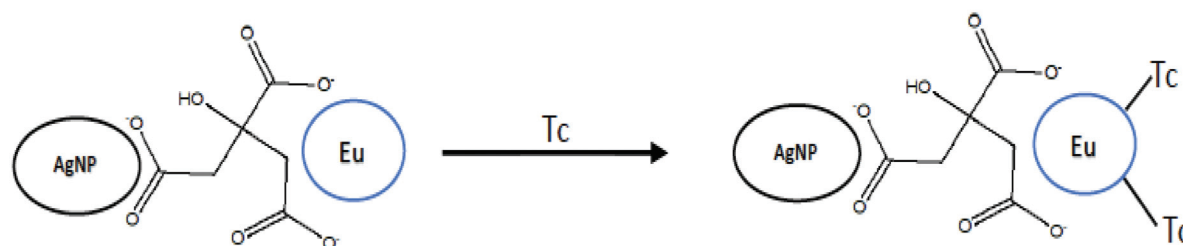
Antipsychotic drug clozapine (purchased from Fluka, St. Louis, Missouri, USA) was electrochemically determined via an Autolab (model PGSTAT 101) using MWCNTs and NPs [5]. Preparing the

Figure 1



Schematic figure of maghemite NP use in dexmedetomidine determination. NP, nanoparticle.

Figure 2



Schematic figure of the detection of tetracycline based on AgNP-enhanced fluorescence of Eu^{3+}. AgNPs, silver nanoparticles.

electrode for detecting clozapine is a multistep process. After polishing with alumina powder slurries, the GC electrode was sonicated with ethanol : distilled water solution (1 : 1 V/V) for 1 min. After that, the bare electrode was scanned in the potential range of 0 : 1.6 V by cyclic voltammetry in the electrolyte of H_2SO_4 (0.5 M) [5]. For the purpose of modifying the electrode, three types of suspension were utilized:

(1) MWCNTs in methanol (MWM): equal amounts of MWCNTs and methanol were mixed together with sonication for 0.5 h.
(2) MWCNTs and WO_3 in methanol (MWWM): equal quantities of MWCNTs and WO_3 NPs were suspended in methanol and sonicated for 0.5 h.
(3) MWCNTs and WO_3 in presence of α-terpinol (MWWT): equal amounts of MWCNTs and WO_3 NPs were suspended in 10% v/v solution of terpinol/methanol and sonicated for 0.5 h.

To prepare the electrode, 1 μl of each of the three suspensions was deposited on the surface of the electrode and was allowed to dry [5].

Tetracycline analysis

It was found that NPs were widely employed in the detection and quantification of tetracyclines (TCs) in urine and milk samples [6], with gold nanoclusters (AuNCs) being used in the determination of TCs using a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) [6], and AgNPs [7] being used to enhance their fluorescence, as the fluorescent carbon NPs were quenched once TCs were introduced [8]. Moreover, Fe_3O_4 magnetic nanoparticles and oleic acid were used for UV–vis spectroscopic determination (obtained via Analytik Jena SPECORD-250spectrophotometer in the range of 190–1000 nm) and voltammetric determination [via Autolab potentiostat/galvanostat (PGSTAT-302N), ECO Chemie, Utrecht, the Netherlands] of TCs purchased from Merck Company (Kenilworth, New Jersy, USA) [9]. As for the preparation of these NPs, various methods were employed. For instance, three types of AuNCs can be synthesized and utilized. The first type (AuNCs@BSA) was prepared by mixing equal volumes of 10 mmol/l of $HAuCL_4$ solution and 50 mg/ml bovine serum albumin (BSA) solution with stirring at 37°C. After 2 min, 1.0 M NaOH (5% of the initial volume of the mixture) was added, and the whole solution was allowed to stand at 37°C for 24 h. The second type (AuNCs@DNA_{C12}) was prepared by mixing 100 μM $HAuCl_4$, 25 μM DNAC12, and 50 mmol/l citrate together and allowing the mixture to stand at 25°C for 24 h. As for the third type (AuNCs@His), it was prepared by mixing 8 ml of 10 mmol/l $HAuCL_4$ and 24 ml of 0.1 M histidine solution, and allowing the mixture to stand for 2 h at room temperature. Eu_2O_3 was dissolved in a small amount of 0.1 M HNO_3 and diluted with water. Then TC was added, and the whole EuTC solution was diluted appropriately to a concentration of 2.5×10^{-5} [6] (Fig. 2).

Another method for the detection of TC was developed. This method employs the AgNPs-enhanced fluorescence of europium Eu^{3+} purchased from Rewin Rare Earth Metal Materials Co. Ltd (Baotou, China) [7]. The NPs for this method were prepared by adding 1 ml of 10 mmol/l silver nitrate and 1 ml of 500 mmol/l citrate to 47 ml of water with continuous stirring, then the mixture was allowed to stand for 10 min at 25°C. After that, the formed silver nitrate was reduced by using 600 μl of freshly prepared sodium borohydride while vigorously stirring the solution. The reduction process was allowed to continue for 30 min before the solution was stored at 4°C overnight to allow for the formation of AgNPs, which is indicated by the change of the solution's color into bright yellow [10]. To prepare Eu^{3+}-AgNPs, an excessive amount of Eu^{3+} was added to the AgNP solution, and then the mixture was centrifuged to remove the unbound Eu^{3+}, and the formed Eu^{3}

$^{+}$-AgNPs were suspended in HEPES buffer by ultrasonic waves [7]. After obtaining the Eu-citrate-stabilized AgNPs solution (Eu^{3+}-AgNPs), the procedure for detecting TC in the analyte starts by preparing different volumes of TC to the Eu^{3+}-AgNP solution, and the volume is completed to 100 μl using HEPES buffer, and then the reaction is allowed to continue for 5 min at 25°C. The fluorescence intensity of the mixture is then measured at 390 nm using a UV-3150 spectrophotometer (Shimadzu, Japan) [7].

Oxytetracycline analysis

Oxytetracycline (OTC) with 99% purity was purchased from Sigma-Aldrich and was determined by Cu@Fe_3O_4. The crystal structure was measured by analyzing the radiograph powder diffraction patterns (M18XHF-SRA; MAC Science, Kanagawa, Japan) [11]. The copper nanoparticles (CuNPs) are prepared by reducing a solution of copper sulfate using sodium borohydride (added dropwise to the copper sulfate at a rate of 10 ml/min) with stirring. The reaction is allowed to continue till the CuNPs are precipitated (which is denoted by the color change of the solution from blue to brown, to black). The solution is then centrifuged at 3000 rpm to separate the NPs. The NPs are then washed with deionized water and methanol, and then dried for 1 day at 60°C [11]. The Cu@Fe_3O_4 core-shell NPs are prepared by first sonicating 0.89 g CuNPs with 5 g iron (III) acetylacetonate for 0.5 h. After that, the mixture is vigorously stirred for 10 min, and then reduced using sodium borohydride (which is added dropwise with vigorous stirring), and the mixture is allowed to stand at 105°C for 0.5 h. After cooling the mixture, the NPs are isolated using a magnet and washed with deionized water, methanol, and ethanol, and dried for 1 day at 60°C [11]. The Fe_3O_4 NPs can be prepared in a similar manner but without using the CuNPs in the process [11]. Furthermore, the oxidative degradation of OTC can be performed using 20-mmol/l hydrogen peroxide in the presence of 0.5-g Cu@Fe_3O_4-CSNs. While the reaction continued, samples from the solution are taken at different times, and the reaction in these samples was stopped immediately using methanol, and the concentrations of both OTC and the hydrogen peroxide are analyzed; the OTC is analyzed using high-performance liquid chromatography (HPLC) (Ultimate 3000; Dionex, Sunnyvale, California, USA), and the hydrogen peroxide using UV/vis spectrophotometer (UV-mini 1240; Shimadzu). Furthermore, the Cu@Fe_3O_4-CSNs were found to be reusable as a catalyst, as they were successfully isolated via a magnet. After washing and drying, they were tested for five times in five subsequent degradation reactions of OTC and were still functional [11].

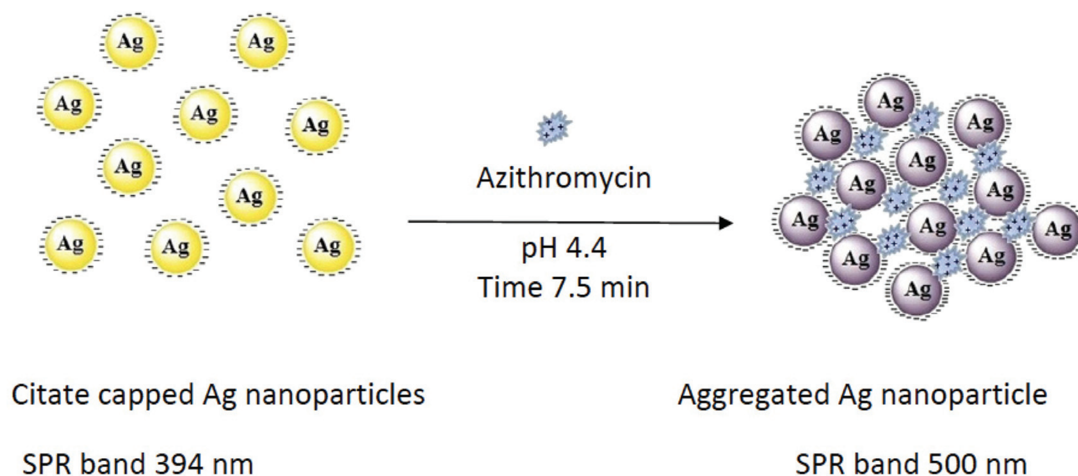
Moxifloxacin analysis

Moxifloxacin (MF) detection depends on the finding that weak chemiluminescence signal intensity of calcein–$KMnO_4$ intensifies by introducing AgNPs in the presence of MF, which is obtained from urine samples, or as therapeutic dosage form (Avolex) or purchased from Sigma-Aldrich [12]. To prepare these NPs, the authors maintained 50 ml of $AgNO_3$ at a constant temperature while stirring in an oil bath for 6 h. Then 60 ml of 1 M NH_4OH in a flask was put in a water bath at room temperature, then the two flasks were exposed to daylight lamp and connected together to allow for transfer of the ammonia gas from the second flask to the flask with the silver nitrate solution, and consequently to allow for the reaction between them to occur and the AgNPs to be formed, and the signal intensity is measured using A Spex (Model F111; SPEX Industries, Edison, New Jersey, USA) spectrofluorometer furnished with a coiled glass flow cell (1.0 mm i.d., 20 mm total diameter) [12]. Furthermore, various methods for enhancement of the analysis of other antibiotics were developed, like ciprofloxacin, which was measured by RP-HPLC at 278 nm using Eudragit RS100 or RL100/PLGA NPs [13]; azithromycin, which was detected by double-beam spectrophotometer using unmodified citrate-capped AgNPs [14,15]; and vancomycin, which was determined using lipid–polymer NPs [16] (Fig. 3).

Acyclovir analysis

NPs have been used for detection of anti-viral drugs. An example of that is acyclovir (ACV) in pharmaceutical formulations, which was determined electrochemically on an AUTOLAB modular electrochemical system (ECO Chemie) using MWCNTs and ZnO NPs [10]. To prepare the electrode for ACV detection, a carbon paste made of graphite powder (55%), ZnO NPs (5%), MWCNTs (10%), and paraffin oil (30%) was prepared and packed into a plastic syringe. Then, a copper wire was inserted through the paste in the syringe to provide an electrical current [10]. The polymer film-modified MWCNTs-ZnO NPs-CPE was fabricated by cyclic voltammetry in the potential range −0.2 to 0.8 V at a sweep rate of 100 mV/s in 0.5 M aqueous $HClO_4$ solution containing 5 mmol/l o-aminophenol in the presence of 5 mmol/l SDS for 25 cycles. The obtained modified electrode (P-o-aminophenol/MWCNTs-ZnO NPs-CPE) was washed with double-distilled water to remove the physically adsorbed material [10]. The

Figure 3



Schematic figure of suggested mechanism for colorimetric sensing of azithromycin upon aggregation of silver nanoparticles.

modified electrode was found to significantly improve the quantification process of ACV [10]. Moreover, it was found that the analysis of several other antifungal drugs was enhanced using NPs, like the detection of clotrimazole using Eudragit RS100 nanocapsules by LC-10A HPLC system (Shimadzu, Japan) at 229 nm [17] and nystatin by using loaded Eudragit RS100/PLGA NPs [18].

Thiamine analysis

Gold nanoparticles (AuNPs) have been used as an indicator of thiamine, whihc results in the formation of a secondary peak in the absorbance spectrum of UV–vis spectroscopy using Perkin Elmer Lambda 25 UV/vis spectrometer [19]. Thiamine was purchased from Nutritional Biochemicals Corporation (Cleveland, Ohio, USA), and the AuNPs were prepared using a 1.03 mmol/l solution of $HAuCl_4$ for the purpose of preparation. Three different concentrations of sodium citrate dihydrate were prepared (40 mmol/l, 20 mmol/l, and 10 mmol/l), and then 6 ml of the previously prepared $HAuCl_4$ solution was added to each of the three concentrations, and the three solutions were heated for 5, 15, and 30 min, respectively [19]. The formed AuNPs were used to prepare several solutions with different concentrations. The solutions prepared were typically formed of 10% volume of the previously prepared AuNP solution, 160 and 120 μM of arginine, tryptophan, and L-glutathione amino acids, along with the target thiamine, using ultrapure water as a solvent [19].

Celecoxib analysis

Many NSAIDs have been detected using NPs, like celecoxib (obtained from Razi Pharmaceutical Company, Cairo, Egypt), which was determined using iron oxide nanoparticles (magnetic) by Perkin Elmer/Lambda 25 UV–vis spectrophotometer (USA), and chromatography separation was recorded using /diode array detector/column C18 [20]. To prepare the MNPs, diluted ammonia was allowed to react with a mixture of $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ for 150 min under nitrogen at 80°C, until the solution turns black. After that TEOS was added to the solution, which was then kept under stirring for 48 h. After that, the formed NPs were isolated using a strong magnet, and washed with water and ethanol, and then dried under vacuum [20]. After that, iron NPs were modified via refluxing them in 3-mercaptopropyltrimethoxysilane (5%) in toluene for 2 days at 90°C, and the resulting modified NPs can be isolated using a magnet and washed with toluene, and then heated to 40°C under vacuum for drying [20]. After modification of the iron oxide nanoparticles, polymers (like the functional monomer AI, and the thermosensitive polymer NVC) were grafted onto the surface of NPs. This can be done by mixing NVC with AI and the NPs in ethanol, then refluxing the mixture with azobisisobutyronitrile for 7 h under nitrogen at 65°C. After that, the grafted NPs were isolated using a magnet then washed with ethanol, and allowed to dry at room temperature [20]. The polymer-grafted NPs were found to be capable of adsorbing the celecoxib molecules efficiently from samples; thus, these polymers can be used for isolation of celecoxib before its determination using HPLC [20].

Indomethacin analysis

Magnetic Fe_3O_4 NPs have been used for extraction/preconcentration of ultratrace amounts of

indomethacin from biological fluids (plasma and urine) before detection by UV-240 Shimadzu spectrophotometer at 310 nm [21]. For this purpose, a mixture of $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ and ammonia solution was allowed to interact together via stirring for 15 min. After discarding the supernatant, the formed iron oxide NPs were washed with deionized water and sodium chloride, and then suspended in deionized water [21]. Measurement of indomethacin using these MNPs was performed by adding CTAB solution to the MNP suspension and adjusting the pH to 10.0, followed by stirring for 5 min, which allows for the CTAB molecules to form a coat on the surface of the MNPs. The MNPs are separated magnetically, and the supernatant is discarded. After that the solution containing the target drug (indomethacin) is subjected to the resulting coated MNPs that absorb the drug molecule on their surfaces. The isolated drug is then available for spectrophotometric detection at 310 nm [21].

Cholesterol analysis

On the contrary, nanoparticles can be used for spectrophotometric determination of some vital substances like cholesterol (which was obtained at a concentration of 3.95 U/mg, from *Pseudomonas fluorescens*). This was achieved by using nanoclay (NCl) in presence of Fe impurity, which acts as the catalyst for reduction of peroxidase at 650 nm [22]. After reaction with TMB in the presence of hydrogen peroxide, peroxidase would produce a characteristic absorption band at 650 nm (measured by Tecan Infinite M200 Pro). However, nanoclay was found to have the ability to simulate the action of peroxidase on TMB, exhibiting a slightly shifted absorption band at 627 nm. The shift can be explained in terms of adsorption of TMB+ on clay [22]. To prepare the electrode for cholesterol detection, nanoclay (NCl) is immersed in a sodium acetate buffer of pH 4.0 for 3 h, then sonicated for 0.5 h to allow for a uniform distribution of the nanoclay in the suspension. Then, the formed suspension was used to cast the previously polished glassy carbon electrode, and the electrode is then allowed to dry at 25°C for 2 h. After that, a solution of cholesterol oxidase was used to cast the NC. After drying the electrode, a layer of poly(3,4-ethylenedioxythiophene) was given and dried overnight. The electrical conductivity and high stability of poly(3,4-ethylenedioxythiophene) makes it suitable as a coat to the electrode, as it would not dissolve in organic solvents, and at the same time prevent the loss of the enzymes from the electrode [22]. Moreover, NC can be used to measure cholesterol amperometrically by applying a potential of −0.5 V, after establishing a constant residual current [22]. Furthermore, cholesterol can be determined using a suspension of NC in acetate buffer (pH 4.0) to which a suitable amount of cholesterol oxidase is added. After that, the cholesterol is added and absorbance measurements are recorded [22].

DNA/RNA detection

In the field of protein and gene expression, DNA-mediated gold-silver nanomushroom has been used for simultaneous stable surface-enhanced Raman scattering (SERS) detection of various DNA and RNA targets [23]. Preparation of DNA-modified AuNPs is performed by mixing a solution of AuNPs with a DNA solution, and the mixture is shaken overnight. After that, a sufficient amount of phosphate buffer is added so that the concentration of phosphate in solution becomes 10 mmol/l, with a pH of 7.4. Then the last step is repeated every 1 h for three times using $NaNO_3$, this time to reach a concentration of 0.1 M $NaNO_3$ after the addition of salting buffer (2 M $NaNO_3$). After that, the solution is shaken overnight and then centrifuged at 7500 rpm for 7 min at 4°C. After decantation of the unmodified DNA, the remaining precipitate is resuspended using 10 mmol/l PB solution. These steps are repeated three times [23]. As for the preparation of DNA and Raman molecule comodified AuNPs, a nonfluorescent small molecular Raman report solution is added to the previously prepared DNA-modified AuNPs and allowed to react for 2 h with mild agitation. After that the mixture is washed with 10 mmol/l PB (pH 7.4) and centrifuged at 7500 rpm for 7 min at 4°C to remove the unreacted Raman reporters. After decantation of the wash solution, the precipitated Raman-DNA-modified AuNPs is resuspended with 10 mmol/l PB buffer [23]. After that, and in order to prepare mushroom-like structures, polyvinylpyrrolidone is added followed by sodium ascorbate. Then a solution of $AgNO_3$ is added to the mixture rapidly with vigorous vortexing for 60 s, and then the mixture is allowed to stand for 30 min, and then washed three times with PB, centrifuged at 7500 rpm for 7 min at 4°C, and resuspended with PB buffer [23]. Detection of DNA/RNA targets is performed by modifying MMPs with streptavidin (SA), and after washing, the capture DNA is added. Then 6 μl of biotinylated capture DNA/RNA is added. Then the solution was shaken for 15 min at 25°C. After that the solution is washed with phosphate buffered saline with tween 20 (PBST). After

washing, equal volumes of the target DNA/RNA are mixed with MMPs at 37°C for 2 h with gentle shaking. After rewashing the solution, functionalized Au-Ag SERS probes are mixed with the solution and allowed to interact with it for 2 h at 37°C under shaking, to avoid aggregates formation. After incubation, the unreacted SERS probes are removed by washing with PBST. After that, SERS detection is performed using XPLORA (HORIBA; Jobin Yvon, France) Raman microscopic system [23].

Protein array detection

ZnS-coated CdSe quantum dot (ZnS/CdSe quantum dots) NP can be used for gene expression, drug screening, and disease detection before fluorescence analysis [24]. To prepare the capture Ab substrate glass, microscope chips were macerated in chrome pickle for 2 h, then washed with water, and dried with nitrogen. After that they were sonicated for 15 min in hexane, acetone, and ethanol and dried at 80°C for 5 min After amino-silanization of the chips' surfaces, they were rinsed with ethyl acetate and used at once [24]. The amino-modified chips can be covered with IgG via soaking them in 5% glutaraldehyde in phosphate buffer saline for 5 h. After that the chips are rinsed with water and dried with nitrogen stream. Then, the rabbit anti-human antibody is added to the surface dropwise. The drops are allowed to stay in contact with the chips for 2 h at 37°C, and it is then washed with PBS. After that, the unreacted aldehyde groups from the glutaraldehyde are blocked using BSA. After that, a final wash is performed using PBS followed by drying under a stream of argon [24]. The immunoassay readout of the antibody on the glass slide and the calculation of the fluorescence intensity were performed using a GSI Scanner Array 4000 (GSI Lumonics) with two lasers (532 and 635 nm) [24]. The results obtained from this method indicate that the quantum dot-labeled biomolecules, as antigens, can be effectively used in protein chips [24].

Streptavidin and immunoglobulin E detection

AgNPs and $AgNO_3$ are used for detection of SA (purchased from Beijing Biosynthesis Biotechnology Co. Ltd, Beijing, China) and human immunoglobulin E (IgE) (obtained from United States Biological, Swampscott, Massachusetts, USA) before analysis by UV–vis spectrophotometer (UV3600; Shimadzu) and spectrofluorometer (RF-5301PC, Shimadzu, Japan) [25]. To prepare the oligo-derived AgNPs (Tag-A), 1 ml of AgNPs with SH-oligomer-Cy5 (75 μl, 10 μM) and SH-oligomer-biotin (25 μl, 10 μM) was incorporated together for at least 18 h. Then the concentration of sodium chloride in the solution was brought up to 0.1 molar using PBS and two molar sodium chloride solutions, and the solution was allowed to stand for 48 h. The conjugate was precipitated via centrifugation for a total of 45 min at 15 000 rpm at 14°C. The resulting precipitate was redispersed in 0.1 M MPBS [24]. As for Tag-B, 1 ml AgNPs with SH-oligomer-Cy3 (75 μl, 10 μM) and SH-oligomer-biotin (25 μl, 10 μM) was incorporated together for at least 18 h, and the same steps performed at tag-A were repeated [25]. Moreover, Tag-C was prepared through incorporating together 1 ml AgNPs with SH-oligomer-Cy5 (75 μl, 10 μM) and aptamer (25 μl, 10 μM) for at least 18 h, and the same steps performed at tag-A were repeated. However, after centrifugation, the resulting precipitate was washed and recentrifuged, and redispersed in 0.1 M PBSM. Tag-C was used for detection of IgE [25]. To detect SA, a modification was performed to a slide using aldehyde, and then a 4×4 microarray was plotted on this slide using 1 mg/ml SA sample and 1 mg/ml BSA (negative control). After that the sample and the negative control were allowed to interact with the slide for 2 h at 37°C, and then the SA was immobilized on the slide via refrigeration. After blocking the slide and washing it with PBST, either Tag-A or Tag-B was allowed to interact with SA for 60 min, then washed with PBST, PBN, and distilled water. Finally, the microarray was scanned after adding FES or silver enhancer solution [25]. The detection of IgE was similar to the aforementioned procedure; a 4×4 microarray was developed on a modified slide, this time using 125 μg/ml anti-human IgE as a sample instead of the SA solution, and the reaction is allowed to continue for 4 h instead of two. After blocking the slide with BSA and washing it with PBST, 30 μl of various concentrations was incubated on the slide for 60 min at 37°C, and then rinsed with PBST. After that, the Tag-C was allowed to interact and bind with IgE for 60 min at 37°C, followed by a washing step. Finally, the microarray was scanned after adding FES [25]. It was found that, this method, which depends largely on a novel silver nanostructure is highly sensitive for protein analysis and can detect concentrations as low as 0.25 ng/ml [25]. Moreover, NPs can be used for determination of a variety of molecules, like anticancer drugs such as gemcitabine, which can be determined by a reversed phase HPLC method using a C18 column and 268 nm UV detection using poly(lactide-coglycolide)/poly-ε-caprolactone alginate [26], and 5-fluorouracil, which can be determined by hydrophilic interaction liquid chromatography/diode

Table 1 Lod of nanoparticles analytical method and Lod of analytical method for different drugs

Drug	Lod of NP analytical method	References	Lod of analytical method	References
L-cysteine	2.3 ng/ml	[2]	0.14 μM	[33]
	2.8 μM	[3]	1.7×10–7 mol/l	[34]
Clozapine	30 nM	[5]	2.08×10^{-10} mol/l	[35]
Tetracycline	4 nM	[6]	5 ng	[36]
Moxifloxacin	5.6 9 10^{-9} M	[12]	0.06 ng/ml	[37]
Acyclovir	0.067 μg/ml	[10]	7.0 ng/ml	[38]
Thiamine	0.5–1 μM	[19]	0.106 μg/ml	[39]
Indomethacin	8.6 ng/ml	[21]	4×10^{-8} g/ml	[40]
5-fluorouracil	11 ng/ml	[27]	50 ng/l	[41]
Morphine	0.15 μg/ml	[28]	5 ng/ml	[42]
Ibuprofen	0.03 μg/ml	[27]	0.8 ng/ml	[43]
Halcinonide	0.04 μg/ml	[30]	0.05 ng	[44]

NP, nanoparticle.

array detector using solid lipid NP and nanostructured lipid carrier [27]. Moreover, citrate-capped AuNPs were used for determination of ibuprofen [27] and opioid drugs such as morphine [28]. Furthermore, morphine, codeine, and methadone have been detected using SDS-coated magnetic Fe_3O_4 NPs by HPLC-UV detection at 220 nm [29]. Corticosteroid drug halcinonide was detected by HPLC-UV detection at 239 nm using polymeric lipid-core NPs and solid lipid NPs [30]. AgNPs can be used for detection of proteins by UV spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and Fourier transform–Raman spectroscopy [31]. Protein kinase activity also has been determined based on various nanomaterials (NPs, nanowires, NTs, nanorods, nanocomposites and semiconductor quantum dots), using different analytical techniques such as colorimetric sensing, resonance-light-scattering sensing, electrochemical sensing, SERS sensing, mass spectroscopy sensing, and fluorescence sensing [32].

The use of NPs in the analysis of the different drug can show higher sensitivity than the traditional analytical methods. Table 1 compared the Lod of NP analytical method and the Lod of traditional analytical method. Tables 2 and 3 show collective data for analyzing different drugs and the used NPs.

Analytical equipment

The analytical equipment mentioned in this article include UV–vis spectrophotometer which was used in the analysis of the drugs of question using the synthesized NPs depending on the absorption patterns of UV–vis light exhibited by these drugs. UV–vis spectrophotometer which was used in the analysis of L-cysteine [2], Dexmedetomidine [4], TC [9], azithromycin [14,15], thiamine [19], indomethacin [21], cholesterol nanoclay [22], SA and IgE [25]. Fluorescence spectrophotometer was used as a mean of quantification depending on luminescence exhibited by photons excitation, and fluorescence spectrophotometer was used in the determination of TC [6]. Electrochemical determination was also employed using potentials measured via three electrode-electrochemical (AUTOLAB modular electrochemical systems) systems, and electrochemical systems as well were used in the determination of clozapine [5], TC [9], ACV [10], and cholesterol nanoclay [22]. HPLC was used in the quantification of the drugs in question depending on the principles of chromatography (normal phase or reversed phase) and using a variety of detectors, including photo-diode array, fluorescence spectrophotometer, and UV–vis spectrophotometer. HPLC was used in the determination of OTC [11], ciprofloxacin [13], clotrimazole [17], nystatin [18], celecoxib [20], gemcitabine [26], 5-flourouracil [27], codeine and methadone [29], as well as halcinonide [30]. Spectrofluorometer also was used in the determination of various drugs by measuring the florescence exhibited by these molecules after electromagnetic excitation; spectrofluorometer was used in the determination of MF [12], whereas spectrofluorometer was used in the determination of SA and IgE [25]. Raman microscopic system was used for the detection and determination of DNA and RNA targets depending on the dispersion or scattering of photons at the same energy as the incident photons. Raman microscopic system was used for the detection of DNA and RNA targets [23].

Nanoparticle characterization equipment

The equipment for the characterization of the synthesized NPs include vibrating sample magnetometer, which was used for determination of the magnetic properties of the NPs [4]. Moreover,

Table 2 Collective data for analyzing different drugs and the used nanoparticles

Drug	Type of NP used	Size of NP	Preparation scheme	Use of the developed NP	Principle of the assay	Sensitivity of the assay
L-cysteine	Multiwalled carbon nanotubes (MWCNT) or silver nanoparticles (AgNPs)	For MWCNT: 30 nm	For MWCNT: pretreatment, followed by polymerization with the aid of AIBN in presence of MAA	Quantitative analysis of L-cysteine	UV–visible spectrophotometer	For MWCNT: the limit of detection is 2.3 ng/ml
		For AgNP: ranges from 5 to 20 nm	For AgNP: a mixture of $AgNO_3$ and PVP is vigorously shaken with drop wise addition of Na-borohydride			For AgNP: limit of detection equal to 2.8×10^{-6} M
Dexmedetomidine	Sodium dodecyl sulfate (SDS) modified maghemite nanoparticles (MNPs)	Around 30 nm	MNPs are prepared, then coated with SDS before being used to adsorb dexmedetomidine	Preconcentration, determination, and removal of trace amounts of dexmedetomidine	Spectrophotometer at 280 nm.	The limit of detection is 3.7×10^{-7} M
Tetracycline	Gold nanoclusters (AuNCs) and AgNPs	For AuNCs: <3 nm	For AuNCs: by using $HAuCL_4$ solution; either with BSA solution, OR DNAC12 and citrate solutions, OR histidine solution	For AuNCs: determination of tetracycline	For AuNCs and AgNPs: florescence	For AuNCs and AgNPs: the limit of detection is 4×10^{-9} M
		For AgNP: around 7 nm	For AgNP: a mixture of $AgNO_3$ and citrate is vigorously shaken, then reduced with sodium borohydride	For AgNPs: enhances the florescence of tetracycline	Spectrophotometer	
Oxytetracycline (OTC)	Copper @Fe_3O_4 core-shell nanoparticles	34.68 nm	Copper nanoparticles are processed with acetylacetonate, then reduced with Na-borohydride	Oxidative degradation of OTC	NA	NA
Moxifloxacin (MF)	AgNPs	14 nm	$AgNO_3$-containing flask is connected to NH_4OH-containing flask at daylight lamp under controlled temperatures to allow reaction and formation of $AgNO_3$	Determination of MF	Introduction of AgNPs increases the weak chemiluminescence signal intensity of calcein–$KMnO_4$	The limit of detection is 5×10^{-9} M

AIBN, azobisisobutyronitrile; BSA, bovine serum albumin; MAA, methyl acrylic acid; MWCNTs, multiwalled carbon nanotubes; PVP, polyvinylpyrrolidone.

atomic force microscopy experiments were carried out to determine the morphology of the synthesized NPs [5]. Moreover, high-resolution transmission electron microscope and high-resolution digital camera were used for imaging purposes [6] as well as the determination of morphology and size of the synthesized NPs [7]. Particle size analysis was also performed by dynamic light scattering (using transmission electron microscope) [14], a laser diffraction particle-size analyzer [18], or photon correlation spectroscopy [13,17], whereas zeta potential values were measured via electrophoretic light scattering using the same instrument [13], using zeta-sizer nano [27] or zeta potential analyzer [11]. Absolute PL Quantum Yield Spectrometer was used for determining the quantum yields [8], whereas scanning electron microscopy [10,18,19] and transmission electron microscopy [10,19] were used for imaging and characterization of the surface structure of synthesized NPs, as well as determination of the size and morphology of the NPs [4]. FTIR spectrometer was used to obtain the FTIR spectrum for the purpose of characterization of the NPs [2,21]. Radiograph diffractometer was used as well for the characterization and determination of the crystal structure of NPs as well as phase characterization of the adsorbent [2,4,21]. GSI scanner array was used for quantification of immunoassays with labeled antibodies [24].

Conclusion

This review highlighted the new approaches of the involvement of nanotechnology in analyzing different

Table 3 Collective data for analyzing different drugs and the used nanoparticles

Drug	Used nanoparticle	Capping material	Size	Assay procedure	Preparation	Sensitivity
Acetaminophen	Copper ferrite-copper oxide (CuO-$CuFe_2O_4$)		Mean diameter of 90 nm	Electrochemical		
Gemcitabine	Poly lactide co glycolide /poly-ε-caprolactone (PLGA : PCL 80 : 20) Alginate		564.7 ±56.5 nm 210.6±6.90 nm	A reverse phase HPLC method using a C18 Luna column (4.6 ×250 mm), 95/5 (v/v) 0.04 M ammonium acetate/ acetonitrile mobile phase (pH 5.5), 1.0 ml/min flow rate and 268 nm UV detection		Accuracy of ~101.45, 94.63, and 97.17% for concentrations 1.9, 9.5, and 38 μg/ml, respectively
5-fluorouracil	Hydrophilic interaction liquid chromatography (HILIC) in combination with diode array detector (DAD)		370 nm	Hydrophilic interaction liquid chromatography (HILIC) in combination with diode array detector (DAD) (250×4.60 mm ID, 5 _m, 200 Ao), Mobile phase is consisting of a mixture of acetonitrile: buffer containing 5 mmol/l ammonium acetate (95 : 5; v/v). The pH of the mobile phase was adjusted to 7.0 using 1 M NaOH. The analysis was carried out at 0.75 ml/min flow rate with a detection wavelength of 265 nm		RSD% values (<2%). The limits of detection and quantification were 11 and 37 ng/ml, respectively
Azithromycin	Unmodified AgNPs	Citrate-capped AgNPs	12 nm	Double-beam spectrophotometer		
Ciprofloxacin	Eudragit RS100 or RL100/PLGA			RP-HPLC 278 nm The mobile phase consisted of 13% (v/v) of acetonitrile and 87% (v/v) of a 0.025 M aqueous phosphoric acid solution, adjusted to pH 3 with triethylamine. The flow rate was set at 1.5 ml/min		
Ceftriaxone	Ag modified-magnetic nanoparticle (Ag-MNPs)			HPLC C18 (125×4.0 mm, 3.5 μm) column, UV detection was set at 270 nm and the flow rate was 1 ml/min		Enrichment factor was obtained 19. The detection limit was 0.02 mg/ml
Clotrimazole	Eudragit RS100 nanocapsules			HPLC-UV–vis Detector column: RP C18 mobile		

(*Continued*)

Table3 (*Continued*)

Drug	Used nanoparticle	Capping material	Size	Assay procedure	Preparation	Sensitivity
				phase: methanol and water (90 : 10 v/v); flow rate: 1 ml/min; wavelength: 229 nm; retention time: 5.1 min		
Nystatin	Loaded Eudragit RS100/PLGA nanoparticles		128 ±6.5–362.9 ±2.6 nm	DSC, SEM, XRPD, and FTIR		
Clozapine	MWCNTs WO_3 nanoparticles		Particle size<100 nm	Atomic force microscopy (AFM) and the electrochemical impedance spectroscopy (EIS). The electro-oxidation of CLZ was investigated on modified glassy carbon electrode by cyclic voltammetry and square wave voltammetry (SWV) methods		Detection limit of 30 nM
Tenofovir	Silver nanoparticles			Square wave adsorptive stripping voltammetry		
Acyclovir	ZnO nanoparticles and carbon nanotube		Average diameter of 20 nm and length in the range of 10–30 nm	Electrochemical determination		Detection limit of 0.067 μg/ml
Cholesterol	Nanoclay			Spectrophotometric determination at 650 nm		For the spectrophotometric (linear detection range 50–244 μM, R2=0.99) and amperometric detection of cholesterol (linear detection range 0.099–1.73 mM, R2=0.998)
Halcinonide	Polymeric lipid-core nanoparticles and solid lipid nanoparticles			High-performance liquid chromatography (HPLC) with the UV detection at 239 nm. isocratic flow rate of 1.0 ml/min, a mobile phase methanol : water (85 : 15 v/v), and a retention time of 4.21 min		
	Gold nanoparticles (AuNPs)	Oligonucleotide	50 nm	Surface-enhanced Raman scattering (SERS)		
	CdSe quantum dot (ZnS/CdSe QDs) nanoparticle	ZnS			Fluorescence analysis	

(*Continued*)

Table3 (*Continued*)

Drug	Used nanoparticle	Capping material	Size	Assay procedure	Preparation	Sensitivity
Protein kinase	Nanoparticles (NPs), nanowires (NWs), nanotubes (NTs), nanorods (NRs), nanocomposites (NCs) and semiconductor quantum dots (QDs),		1–100 nm			Colorimetric sensing Resonance-light-scattering sensing Electrochemical sensing surface-enhanced Raman spectroscopy (SERS) sensing Mass spectroscopy (MS) sensing Fluorescence sensing
Various proteins and enzymes	AgNP				Ultraviolet (UV) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and Fourier transform–Raman spectroscopy UV–vis spectroscopy analysis was done to confirm the presence of silver nanoparticles range of 300–700 nm	
Celecoxib	Iron oxide NPs (magnetic)			Grafting of nanoparticles	UV–vis spectrophotometer Chromatography separation was recorded using /diode array detector/column C18, 15 cm, 4.6 mm, 5 mm The SEM micrographs was used for morphology measurement. Thermogravimetric analysis	Recovery of more than 95% was obtained
Ibuprofen-poly (methyl-methacrylate	Ultrafine nanoparticles	Mean diameters smaller than 20 nm			Transmission electron microscopy (STEM) UV–vis spectrophotometry quasielastic light scattering (QLS)	
Ibuprofen	AuNPs	Citrate capped		Size of synthesized AuNPs and the aggregated forms were monitored by infrared (IR) spectroscopy and transmission electron microscopy (TEM), colorimetric determination partial least square (PLS) regression		Detection limits of 0.03 μg/ml (S/N=5)
Indomethacin	Magnetic Fe_3O_4 nanoparticles	Mean diameter of 9±1.3 nm		The UV–vis spectrophotometry transmission electron microscope (TEM) was used to characterize the size and morphology of the nanoparticles The	Using cetyltrimethylammonium bromide (CTAB) adsorbed onto the surface of magnetic Fe_3O_4 nanoparticles	Limit of detection of 8.6 ng/ml, enrichment factor of 99 and the relative standard deviation (%RSD) of 1.9% (for a concentration of 50 ng/ml) Good recoveries

(*Continued*)

Table3 (*Continued*)

Drug	Used nanoparticle	Capping material	Size	Assay procedure	Preparation	Sensitivity
				Fourier transform infrared (FTIR)		(89–102%) with low % RSD, (1.7–2.7%)
Morphine	AuNPs	Citrate-capped		Size of synthesized AuNPs and the aggregated forms were monitored by infrared (IR) spectroscopy and transmission electron microscopy (TEM), colorimetric determination partial least square (PLS) regression		Detection limits of 0.15 μg/ml (S/N=5)
Morphine, codeine and methadone	Sodium dodecyl sulfate (SDS)-coated MNPs MNPs: Fe3O4 NPs	SDS	7 nm±1.5	HPLC Cyano (4.6 ×250 mm, 5 μm) analytical column was used. The injection volume was 20 μl and the mobile phase was acetonitrile: sodium flow rate of 1.0 ml/min with isocratic elution and UV detection was performed at the wavelength of 220 nm	Extraction/ preconcentration	Recoveries were 91, 94 and 98% for morphine, codeine and methadone, respectively enrichment factors (EFs) of 91, 94, and 98 for morphine, codeine and methadone, respectively, and the limits of detection were <0.27 ng/ml
Acetazolamide	Eudragit RL100 nanoparticles		<200 nm in size	Kinetic study IOP was measured using a Riester Tonometer in rabbit's eye Transmission electron microscopy Fourier transform infrared spectroscopy		
Streptavidin (SA) and human IgE	AgNPs		Diameter was D=24.5 ±4.7 nm	Microarray det UV–vis spectrophotometer		Limit of detection is determined to be 0.25 ng/ml
	AgNPs			UV spectroscopy, Fourier transform infrared (FTIR) spectroscopy and Fourier transform–Raman spectroscopy		
Thiamine	Gold nanoparticles		20–30 nm	UV/visible spectrophotometry		

AgNPs, silver nanoparticles; AuNPs, gold nanoparticles; IgE, immunoglobulin E; MWCNTs, multiwalled carbon nanotubes; SEM, scanning electron microscopy.

drugs, antibiotics, antivirals, and amino acids, besides it showed that nanotechnology involvement in drug analysis can solve some of the old problems.

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There are no conflicts of interest.

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