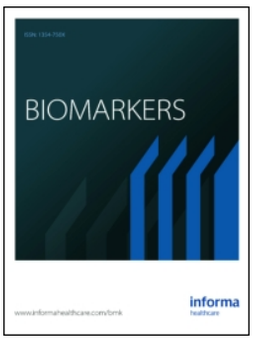

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Quantitative analysis of sarcosine with special emphasis on biosensors: a review

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Abstract

The quantitative determination of sarcosine is of great importance in clinical chemistry, food and fermentation industries. Elevated sarcosine levels are associated with Alzheimer, dementia, prostate cancer, colorectal cancer, stomach cancer and sarcosinemia. This review summarizes the various methods for quantitative analysis of sarcosine with special emphasis on various strategies of biosensors and their analytical performance. The current biosensing methods have overcome the drawbacks of conventional methods. Sarcosine biosensors work optimally at pH 7.0 to 8.0 in the linear range of 0.1 to 100 μM within 2 to 17s and and between 25 and 37°C, within a limit of detection (LOD) between 0.008 and 500 mM. The formulated biosensors can be reused within a stability period of 3-180 days. Future research could be focused to modify existing sarcosine biosensors, leading to simple, reliable, and economical sensors ideally suited for point-of-care treatment.

Keywords: Sarcosine biosensor, Sarcosine, Sarcosine oxidase, Prostate cancer

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Introduction

Sarcosine, a non-proteinogenic amino acid derivative, also known as N-methylglycine, occurs in the body as a product of the metabolism of glycine and creatine. It shares properties with both glycine and D-serine (Porter et al., 1985). Various physio-chemical properties of sarcosine are depicted in table 1 (Strzelecki et al., 2015).

Sarcosine is synthesized by the glycine N-methyltransferase (GMT) and metabolized by sarcosine dehydrogenase (SARDH) enzyme (Heger et al., 2016). An overview of sarcosine metabolism as well as various biological functions of sarcosine is represented in figure 1 [near here]. Sarcosine is present in a variety of food sources viz egg yolks, legumes, nuts, vegetables, turkey, ham, and other meats (Allen et al., 1993). In the human body, it is present in skeletal muscles and prostate. Sarcosine represents an essential role in methyl donation, homeostasis and the one-carbon cycle. It is a major factor in nucleic acid synthesis. It accelerates the cell division and therefore helps in the growth and development of the fetus (Fasman and Blout 1963). Sarcosine has a distinct mild sweet flavour. An ingredient of toothpaste for decades, it prevents cavities and causes foaming. The increased levels of sarcosine have been implicated in pathophysiology of multiple diseases like Alzheimer, dementia, prostate cancer, colorectal cancer, stomach cancer, sarcosinemia, dimethylglycine dehydrogenase deficiency, eosinophilic esophagitis and Lewy body disease (Soliman et al., 2012, Bar-Joseph et al., 2012, Tsuruoka et al., 2013). It is also a promising biomarker for prostate cancer. Research during past decades uncovered the clinical benefits of sarcosine in treating mental health disorders, including schizophrenia, depression, obsessive-compulsive disorder, seizures and stroke (Wu et al., 2011). Serum sarcosine level is normally 80.8ng/mL and 102.3ng/mL in females and males respectively

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(Strzelecki et al., 2015). Various physico-chemical properties of sarcosine are summarized in table 1 [near here].

The defect in sarcosine metabolism causes multiple chronic conditions. These concerns have led the researchers to design various analytical devices to detect sarcosine. The most common techniques used for sarcosine determination are enzymatic assays (colorimetric or fluorometric), gas chromatography (GC), liquid chromatography (LC), mass spectrometry (MS), LC/MS, liquid chromatography coupled to mass spectrometry (HPLC/MS) or HPLC/UV, capillary electrophoresis and capillary electrophoresis with fluorescence detection etc. (Valenti et al., 2015, Gray et al., 2017, Bohm et al., 2012). Enzymatic colorimetric/fluorometric methods are time-consuming and expensive. The chromatographic separation techniques using MS as detector have a high cost, whereas fluorescence detection such as CE-LIF requires an additional derivatization step (Liu et al., 2019, Pundir et al., 2019). Further, these methods possess some major drawbacks like the high-cost instrumental setup, expertise in operating the instruments, reagent and sample volumes consumption, sample pre-treatment and analysis time procedure (Maggio, 2018). To overcome these drawbacks of traditional methods, biosensors have been designed. The biosensor methods are highly specific, sensitive, cheap, rapid and reproducible (Turner 2015). Recently, the analytical performance and electron kinetics of electrochemical biosensors have been improved using nanomaterials in the designing process of biosensors (Harrad et al., 2018). This review provides a comprehensive knowledge about various analytical methods of sarcosine with special emphasis biosensors, their classification, principles, merits, and demerits. Future perspectives have also been discussed to improve biosensing technology in terms of cost and real-time monitoring. To the best of our knowledge, this is the first-time sarcosine biosensors have been reviewed.

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Clinical significance

• Elevated sarcosine levels are associated with prostate and colorectal cancer, Alzheimer, dementia, stomach cancer and sarcosinemia.

• Quantitative determination of sarcosine is of great importance in clinical chemistry as well as food and fermentation industries.

• Attempts made in development of sarcosine biosensors have been reviewed with their advantages and disadvantages, so that scientist and clinicians can improvise the methods of developing more potent sarcosine biosensor applicable in multitudinous fields.

• This is the first comprehensive review which compares the various immobilization methods, sensing principles, strategies used in biosensors and their analytical performance in detail.

Conventional method for sarcosine analysis

Reported traditional methods for sarcosine analysis involves titrimetric, spectrophotometry, turbidimetry, ionization mass spectrometry, pulsed amperometry, automated amino-acid analysis, capillary electrophoresis, high performance liquid chromatography (HPLC), Hydrophilic interaction liquid chromatography electrospray tandem mass spectrometry (HILC-ETMS), reverse phase HPLC and chemiluminescence. The conventional methods now available for sarcosine determination lack specificity.

Potentiometric titration

Principle: Potentiometric titration analysis involves the measurement of electric potential of indicator electrode with respect to reference electrode as a function of titrant volume represented as: Reference electrode [E_{ref}]//analyte solution//indicator electrode [E_{ind}] (Balson and Lawson

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1935). Fang Y et al., in 1998 quantified sarcosine by potentiometric titration with sodium hydroxide. This method optimally detected 1~15 g/L concentration of sarcosine (Xia and Hou, 1998).

Merits: inexpensive, require no indicator

Demerits: pH sensitive, require solution preparation, time-consuming and inaccurate.

Enzyme coupled colorimetric method

Principle: A specific enzyme coupled colorimetric assay was developed for quantification of sarcosine in urine. This method is based on formation of hydrogen peroxide (H_2O_2) via sarcosine oxidase (SOx). The liberated H_2O_2 reacts with Amplex Red to produce resorufin, a red colored fluorescent product, which was measured spectrophotometrically. The limit of quantification (LOQ), detection (LOD) and linearity coefficient were found to be1 µM, 0.7µM and 0.990 respectively. No interference was reported from glucose, ascorbic acid and bilirubin (Yamkamon et al., 2018).

Merits: Rapid, specific, no requirement of complicated apparatus and easy to perform.

Demerits: Low sensitivity, false-positive readings, not applicable in turbid samples.

Chromatographic methods

Various chromatographic methods were used to determine sarcosine levels.

Ion-exchange liquid chromatography (IELC)

Principle: An ion-exchange liquid chromatography with ninhydrin post column derivatization and double channel visible detector was used. Glass column was filed with strong cation

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exchanger in sodium cycle. Buffer eluted fractions of sarcosine were collected by automated fraction collector with 0.25 ml/min flow rate. The temperature range and pH range of column was 35 -95°C and 5.5 respectively. Tin chloride ($SnCl_2$) was used as a reducing agent. The prepared solution of ninhydrin was stored under inert atmosphere (N_2) in dark at 4°C. The elution of sarcosine was done by buffer containing 10.0 g of citric acid, 5.6 g of sodium citrate, and 8.36 g of NaCl per litre of solution and pH of buffer was 3.0 and flow rate at 0.25 ml/min. Reactor temperature was 120 °C. The fractions were collected by automated fraction collector. The LOD of sarcosine obtained using post-column derivatization with photometric detection was1.59±1.08 µM. The detection was further optimized by UV-VIS spectrometer analysis, which detected more promising LOD of 1.7 µM (Cernei et al., 2012).

Merits: Low-cost, robust and simple

Demerits: Low sensitivity, requirement of column modifications and optimization, insufficient LOD.

Liquid chromatography with tandem mass spectrometry

Principle: Chromatographic separations of the analytes were done by phenyl-hexyl column with 0.1% formic acid in water and acetonitrile, separately, under a gradient program.

Jiang Y et al., in 2010 was reported a method for quantitative determination of sarcosine and related compounds in urinary samples by liquid chromatography with tandem mass spectrometry. The linear range of the analytes was from 0.003 - 40 µmol/L. The LOD of location was from 0.05-4 nmol/L and the LOQ ranged from 3 - 20 nmol/L. The correlation coefficients were between 0.9986 and 0.9999 i.e. R^2>0.99, which indicated good linearity. The LOQ of this method was in the range, 3 to 20 nM (Jiang et al., 2010).

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Merits: Simple, fast, sensitive, choice of stationary phase and columns and can detect down to part per million (ppm) levels for many compounds.

Demerits: Expensive, requirement of skilled persons to operate, sample preparation and interferences.

Gas chromatography–mass spectrometry

Principle: Sarcosine was determined directly using hexyl chloroformate derivatization followed by direct immersion solid-phase micro extraction and fast gas chromatography-mass spectrometric (GC-MS) analysis. The procedure utilized a narrow ionic liquid bore column and was automated by customized configuration of the utilized XYZ robotic system which allowed a LOQ of 0.06 µg per litre for sarcosine. A cut-off threshold value of 179 µg sarcosine obtained 79% sensitivity and 87% specificity with $p<0.0001$ (Bianchi et al., 2011).

Merits: Fully automated, non-invasive, rapid and directly analyze gas samples/volatile analytes, sensitive.

Demerits: Often expensive and time-consuming.

High-Performance Liquid Chromatography

Principle: A sensitive method for sarcosine analysis, based on fluorometric detection of derivatizing sarcosine was developed. The separation was achieved with gradient elution on a hexyl-phenyl column using sodium acetate buffer and tetrahydrofuran as the mobile phase. The method showed a LOD of 8.9 ng/mL and a determination range of 0.5-20.0 µM (Chung et al., 2015).

Merits: Faster speed, higher resolution, greater sensitivity and increased precision.

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Demerits: Requirement of a precolumn and cost.

Liquid chromatography-Coulometric detection

Principle: Determination of sarcosine as a potential tumour marker by liquid chromatography with coulometric detection. Zitka et al., in 2011 optimized a fast method for determination of sarcosine in urine using a chromatographic method including C18 reverse phase column. For proper separation of sarcosine, methanol was used as non-polar component of mobile phase and trifluoro acetic acid (80 mM) was used as ion pairing agent. This method was able to determine ultra-low concentrations of sarcosine as using four-channel coulometric electrochemical detection. The LOD was found to be 1.0 pg/ml in 30 minutes (Zitka et al., 2014). Merits: The method is fast and can detect analyte at its lowest concentration.

Demerits: The method is restricted to only liquid phase soluble compounds only and time consuming.

Spectrophotometric methods

Principle: The simplest and most widely available technique for determination of sarcosine, generally involves the formation of colored compounds for subsequent absorbance measurements (200–800 nm). Cernei et al., in 2012 was reported a method for spectrometric and electrochemical analysis of sarcosine as a potential prostate carcinoma marker. Spectroscopy was done by highest yield of the derivatization reaction of ninhydrin, as done for amino-group containing compounds. Absorption spectra of the glycine-ninhydrin and sarcosine-ninhydrin reaction showed in the range, 400 to 800 nm and there was no shift in the absorption maximum. Optimised conditions, concentration was showed from 1 to 50 μM. The calibration curve exhibited a good linearity with correlation coefficient $R^2 = 0.994$ (Cernei et al., 2012).

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Merits: Simple and fast analysis.

Demerits: Analytical interference, slow color development, and low specificity.

Mass fragmentographic method

Principle: Mass fragmentography was combined with push-pull perfusion techniques for measurements of sarcosine and other amino acids released in vivo. Amino acids extracted from brain perfusate were converted to N-pentafluoropropionyl (PFP) hexafluoroisopropyl (HFIP) derivatives and detected by mass fragmentogram. Gas chromatographic separation of the amino acid derivatives is achieved on a packed glass column filled with trifluoropropylsilicone as stationary phase. The LOD for sarcosine ranges from 50 femtomol to 1 picomol (Wolfensberger et al., 1982).

Merits: High precision, provide structural specific detection, speed of the derivatization procedure, small sample size

Demerits: Limited morphological information, compound must be thermostable, expensive, more suitable for drugs.

Emerging techniques for detecting sarcosine

Several distinctive drawbacks such as time-consuming, non-specificity, required technical personnel and expensive instrumentation set-up and chemicals exhibited by the conventional methods. Therefore, efficient and convenient techniques were developed for the rapid detection of sarcosine in various biological materials (serum, feces, urine). As sarcosine biosensor is used for electrochemical detection of various diseases like Alzheimer, dementia, prostate cancer, colorectal cancer, stomach cancer, sarcosinemia, dimethylglycine dehydrogenase deficiency,

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eosinophilic esophagitis and Lewy body disease. The study of amperometric sarcosine biosensors is very limited. Table 2 summarizes the reported electrochemical methods revealing the electrode used, immobilization method, linearity, temperature, pH, potential, sensitivity, linearity, LOD and their applications.

Concept of Biosensor

Biosensor is an electroanalytical device which is used to detect and monitor the specific analyte present in biological samples by producing an electrical signal, which is proportional to the quantity of measured analyte (Uniyal and Sharma, 2018). **[**figure 2 near here**]**

Numerous techniques have been developed in designing of sarcosine biosensors. Among them, nanomaterials-based biosensors have drawn special attention, due to their high sensitivity, specificity, rapid response time, ease of procedure, cost and reusability. The biosensors are widely used in medical field, diagnostics, food industry, agriculture industry, dairy sciences, metabolic engineering, drug production, quality control, marine, mining, military and biodefense sector (Neethirajan et al., 2018, Veloso et al., 2012). Incorporation of nano-materials in biosensors provides striking opportunities for enhancing electrochemical, mechanical, optical and magnetic properties for building new generation biosensor technologies (Hou et al., 2016).

Classification of sarcosine biosensors

A number of criteria like bioreceptors, mode of signal transduction, immobilization technique can be used for classification of sarcosine biosensors. Based on transducers, sarcosine biosensors reported till date, can be classified into 6 types: i) amperometric ii) potentiometric) iii) impedimetric iv) photoelectrochemical (PEC) and v) immunosensor. Table 2 represents

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summary of various electrochemical biosensors for determination of sarcosine. [Insert table 2 near here].

Amperometric biosensors

Amperometric sensors measure current change resulting from oxidation/reduction of an electroactive species in a biochemical reaction, while a constant potential is being applied. The change in current is related to the concentration of the species in solution (Jackowska et al., 2012).

SOx/carboxylated multi-walled carbon nanotubes (c-MWCNT)/Chitosan/copper nanoparticles (CuNPs)/gold (Au) based biosensor:

Narwal et al., in 2018 covalently immobilized SOx onto the surface of an Au electrode pre-coated with electrodeposited c-MWCNT)/Chitosan/Cu NPs. The fabricated biosensor was then used to detect sarcosine in blood serum of prostate cancer patients. The response time of the sensor was 2s with high sensitivity of 277.5 μA/μM/cm^2, low LOD of 0.1 pM and better storage stability of 180 days at 35°C. The analytical recoveries of adding sarcosine in serum at 0.5μM and 1.0 μM sarcosine concentration were 95.5% and 97.30 respectively. The precision i.e. within and between-batch coefficients of variation (CVs) were 1.08% and 1.70% respectively. A good correlation (R^2=0.99) with 3.4% precision was recorded. The effect of interferences (citric acid, glucose, glutamic acid, urea and ascorbic acid) was also evaluated which showed practically no effect on biosensor response. The working of biosensor was based on the interaction of sarcosine with the immobilized sarcosine oxidase (SOx) leading to production of H_2O_2 as represented below (Narwal et al., 2018).

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{SOx}} \text{Glycine} + \text{Formaldehyde} + H_2O_2 \xrightarrow{\text{HRP}} 2H^+ + O_2 + 2e^-$$

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SOx/nano platinum (Pt)/zeolitic imidazolate framework-8 (ZIF8) powder/glassy carbon electrode (GCE) based electrode:

Recently, Yang et al., 2018 prepared a modified enzymatic amperometric biosensor for the determination of sarcosine in human urine by immobilizing nano Pt and ZIF8 powder onto GCE. The linear range, sensitivity and detection limit were 5 to 30 μM, 4.05 nA μM^{-1} and 1.06 μM (S/N= 3) respectively. The shelf-life of the biosensor was 3 days with 60 s response time (Yang et al., 2018).

SOx nanoparticles/ gold (Au) based electrode:

In another study, sarcosine biosensor was formulated by covalently attaching sarcosine oxidase nanoparticles onto polycrystalline Au electrode (Kumar et al., 2018). It was employed for determination of sarcosine in serum patients with prostate cancer compared with their controls. The biosensor worked at an optimum potential of 1.0 V, pH 6.5 and temperature 35 °C within 2 seconds. The biosensor response obtained a linear relationship between sarcosine concentrations in the wide range of 0.1-100 μM and displayed a limit of detection of 0.01 μM. Interference study with urea, citric acid, uric acid, glutamic acid and ascorbic acid, did not change the response of biosensor. The sensor reported an excellent storage stability of 180 days. The serum analytical recovery of sarcosine was 94.22 % and 97.81 % at levels of 0.5 μM and 1.0 μM respectively. In addition, the sensor also reported the coefficients of variations (CVs) as 0.083% (within batch) and 0.067 % (between batch).

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SOx/ within polyvinyl alcohol (PVA)/partially prehydrolyzed tetraethyl orthosilicate matrix (PVA-TEOS) / graphite electrode (GE):

Lad et al., in 2014 constructed a sensitive and robust biosensor involving immobilization of sarcosine oxidase within polyvinyl alcohol (PVA) and partially prehydrolyzed tetraethyl orthosilicate matrix (PVA-TEOS) at the surface of an "in-house" fabricated graphite electrode (GE). The sensor exhibited enhanced sensitivity 4.0131×10^{-5} Amps cm^{-2} mM^{-1} with a slightly higher response time of 17s. The biosensor response was linear from 0.5-7.5 mM with a detection limit of 0.5 mM. The sensor exhibited a excellent reproducibility of 0.84% with higher stability (Lad et al., 2014).

Critical analysis of amperometric biosensors

Here, the biosensors based on the SOx/ c-MWCNT/Chitosan/CuNPs/gold (Au) showed promising detection limit due to high absorption capacity of the c-MWCNT, uniform distribution of the enzyme by chitosan layer, high biocompatibility of the CuNPS and Au electrode. Moreover, nano Pt/ZIF8 powder/GCE exhibit high sensitivity due to good electrical properties of the GCE. In addition, Kumar et al., 2018 showed effectiveness of SOxNPs and exhibited an excellent time response which is comparable with the Narwal et al., 2018. Furthermore, Lad et al., study based on graphite electrode displayed high reproducibility due to high surface area of the graphite electrode.

Potentiometric sarcosine biosensors

Potentiometry, one of the oldest instrumental methods, has well-established position in the sensorics analysis. This analytical technique is an attractive tool for many practical applications as it allows the determination of various ions in the wide range of concentrations and employs inexpensive measurement equipment (Koncki, 2007).

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Screen printed electrode (SPCE) based potentiometric biosensor

Rebelo et al., in 2014 characterized a cost-effective potentiometric enzyme biosensor based on the covalent immobilizated SOx on the surface of the screen-printed carbon electrode, using EDC and NHS for detection of sarcosine concentrations in synthetic urine samples. The sensor had dynamic linear range of 10–100 nM, a low limit of detection of 16 nM and storage stability of 60 days at 4°C. The biosensor exhibited a linear relationship for sarcosine concentrations ranging from 10 nM to 0.1 mM, with a correlation coefficient 0.9966 (Rebelo et al., 2014).

GCE based potentiometric biosensor

Revin et al., in 2015, for the first time, employed 3-amino-5-mercapto-1,2,4-triazole (p-AMTa) GCE to fabricate a potent biosensor for selective determination of sarcosine in presence of elevated levels of uric acid and ascorbic acid. The response time for was <40 s with a linearity range from 0.1 mM to 8.0 mM. It showed a voltammetric signal for sarcosine oxidation at 0.96 V (Revin et al., 2015).

Merit: Simple, cost effective, highly selective and sensitive,

Demerit: Use of synthetic samples.

Critical analysis of potentiometric biosensors

SPCE has an encouraging role in the potentiometric sarcosine biosensor as compared to the GCE. SPCE exhibits low detection limit, high linear range, rapid response and high stability as compared to the GCE. It might be due to low background noise and broad potential window of SPCE as compared to the GCE.

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Impedimetric sarcosine biosensor

The impedimetric biosensor uses immobilizing bioreceptors at the surface of electrode. The binding ability of bioreceptors is verified through the detection of either shift in impedance or change in capacitance at the electrode interface (Seven B et al., 2013).

Canbay et al., developed aptamer based impedimetric biosensor for determination of sarcosine in urine samples. The operation of the biosensor was based on specific interaction between sarcosine specific reduced DNA aptamer and sarcosine using electrochemical impedance spectroscopy (EIS). A graphene oxide assisted systemic evolution of ligands by exponential enrichment (GOSELEX) system was incorporated for selecting DNA aptamer specifically.

The gold electrode was incubated with 1 μM of aptamer and 1 mM of 6-mercapto-1-hexanol (MCH) for 17 hours and 1 hour respectively at 37° C. The EIS measurements were recorded at a potential of 0.223 V, an amplitude of 0.01 V and frequency range from 10 kHz to 100 mHz, A linear detection range between 5 pM and 500 nM of sarcosine was achieved (Canbayi et al., 2015).

Merit: Label-free, simple, reliable, specific, sensitive and specific, reproducible.

Demerit: More response time, expensive and skilled person to operate.

Photoelectrochemical (PEC) sarcosine biosensor

A Photoelectrochemical (PEC) sensor is fabricated on photoactive electrodes, which can convert photoirradiation to electrical signal. Various photoactive materials were metal-contained semiconductors, such as TiO_2, Graphitic carbon nitride (g-C_3N_4), CdSe, CdTe, ZnO and ZnS, etc (Zang et al., 2017). A novel photoelectrochemical sensing platform based on $CuInS_2$ based photocathodic enzyme sensing was formulated for sarcosine determination. The heterostructure copper indium disulfide (CuInS2) microspheres, 3D NiO nanofilm and indim tin oxide (ITO)

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were successfully synthesized as a photoelectrode material. This resulted (CuInS2/NiO/ITO) complex was then then coupled to SOx. The O_2 dependent cathodic photocurrent was suppressed due to the competition between the O_2 sensitive photocathode and SOx towards O_2 reduction. The sensor had a wide linear range from 0.01-1.00 mM, with an LOD of 0.008 mM (S/N=3) and correlation coefficient of 0.995 using linear regression equation $I=3.814e^{-8}c-1.122e^{-8}$. Ascorbic acid, dopamine, lysine, histidine, urea and glucose at 0.1 mM concentration showing no interference (Jiang et al., 2018).

Reidel et al., in 2013 employed the CdSe/ZnS quantum dots (QDs) for fabrication of PEC biosensor. SOx were immobilized on the QDs by means of the polyelectrolyte poly allylamine hydrochloride (PAH). By using SOx, the photocurrent was suppressed because of biocatalytic oxygen reduction. Multi-layer systems were built up to six bilayers through electrostatic interactions. The $[SOD/PAH]_{6-}$ layer system resulted in a signal change of $0.041\%Um^{-1}$. The linear range is between 100 uM and 1 uM sarcosine and higher sensitivity of $0.041\%uM^{-1}$ with Km value of 1 mm (Reidel et al., 2013).

Merit: Remarkable selectivity, sensitivity, inherent miniaturization, good anti-interference capability and easy integration.

Demerit: Use of photoactive material.

Immunobiosensor

Immunosensor is affinity ligand-based sensor, which worked on the basis of specificity of the molecular recognition of antigens by antibodies to form a stable complex. The immunosensing applications are now used in medical diagnosis, clinical analysis, environmental and food quality control (Luppa et al., 2001).

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An ultrasensitive FRET based biosensor for the detection of sarcosine was developed by Heger et al., 2015 using sandwich immunoassay in prostatic cell lines (PC3, 22Rv1, PNT1A) as well as in urine samples of prostate adenocarcinoma patients (Heger et al., 2015)**. [**figure 3 near here]

It utilized nanomaghemite core based paramagnetic nanoparticles surface as immobilization support for anti-sarcosine antibodies. The detection was based on spectral overlap between gold-functionalized green fluorescent protein (GFP) and antibodies @quantum dots bioconjugate (λex 400 nm). Based on FRET efficiency (F604/F510 ratio) the sarcosine curve was tested. The sarcosine was determined with detection limits of 50 pM, linear dynamic range (5–50 nM) and an overall assay time of 30 min.

Merit: Lesser analysis time, increased sensitivity and simplification and automated.

Demerit: Interference and the low applicable range.

Conclusion and future perspectives

This review has highlighted the various biosensors developed till date for detection of sarcosine in biological samples. Compared to other conventional methods, biosensors are highly selective, sensitive, efficient, automated and rapid. The nanomaterial-based biosensor exhibited better analytical performances in terms of working sensitivity and potential detection limit. Among the available electrochemical sarcosine biosensors, amperometric biosensors are dominating.

Future work may focus on clarifying the mechanism of interaction between nanomaterials and biomolecules on the surface of electrodes or nanofilms and using novel properties to fabricate a new generation of biosensors. Use of protein nanoparticles could be employed to construct of highly sensitive sarcosine biosensors. Despite the development of different types of sarcosine detecting biosensors, no sarcosine biosensor is commercially available at the present time. Research could be focused on launching a commercial sarcosine biosensor**.**

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Table 1: Physiochemical properties of sarcosine

Molecular Formula	C3H7NO2
Appearance	Deliquescent crystals or powder
Molecular framework	Aliphatic acyclic compounds
Melting point	208 to 212 °C
Boiling point	195.1 °C
Solubility	89.09 g L−1 (at 20 °C)
Density	1.1±0.1 g/cm
Molecular weight	89.094 g/mol
Enthalpy of vaporization	47.5±6.0 kJ/mol
pKa	2.36
Polarizability	8.66 $Å^3$
Polar surface area	49.33 $Å^2$
Refractivity	20.78 $m^3 \cdot mol^{-1}$
Physiological charge	0
Clinical significance	treatment of schizophrenia, depression, Alzheimer, marker of prostate cancer
Biofluid location	Serum, plasma, Cerebral spinal fluid, urine, faeces

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Table 2: Comparison of various electrochemical biosensors for determination of sarcosine.

Type of biosensor	Support of Immobilization	Method of immobilization	LOD	Linearity	Sensitivity	pH	Temp	V	Response time	Storage (days)	Application	Reference
Amperometric	MWCNT/ Chitosan/ CuNPs/AuE	Electrodeposition	0.1 pM	0.1-100 µM	277.5 µA/µM/cm^2	7.0	35 °C	0.2	2 s	180	sera of prostate cancer	Narwal et al., 2018
Amperometric	Pt@ZIF8/ GCE	Physical adsoprtion	1.06 µM	5 to 30 µM	4.05 nA µM^{-1}	7.0	NR	0.4	NR	3	urine	Yang et al., 2018
Amperometric	SOxNPs/AuE	Covalent immobilization	0.01 µM	0.1-100 µM	NR	7.5	35 °C	1	2s	180	urine	Kumar P et al., 2018
Amperometric	PVA-Ag/AuNPs-pphTEOS-SOD/GE	Physical adsoprtion	0.5 µM	0.5-7.5 µM	4.0131 × 10^{-5} Amps cm^{-2} mM^{-1}	8	NR	0.55	17s	NR	Aqueous media/FMCA	Lad et al., 2014
Potentiometric	p-AMTa/GCE	Electrodeposition	NR	0.1-8.0 µM	NR	7.2	NR	0.96	NR	NR	human fluid	Revin et al., 2015
Potentiometric	SOX/EDC/NHS/Au/ ZnONPs/S	Covalent immobilization	16 nM	10–100 nM	NR	7.2	25° C	0.6	NR	60 days	synthetic urine	Rebelo et al., 2014

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	PEs											
Impedimetric	DNA aptamer/TCEP/MCH	physical	500nM	5pM	NR	7.5	NR	0.223	NR	NR	synthetic urine	Canbay et al.,
Photoelectrochemical	CuInS2/NiO/ITO photocathode	hydrothermal method	0.008 μM	0.01-1.00 μM	NR	7.4		0.0	NR	NR	NR	Jiang XY et al., 2018
Photoelectrochemical	CdSe/ZnS/SOD/QD	electrostatic interactions	NR	100-1 μM	NR	NR	7.6	NR	NR	3days	serum	Reidel et al., 2013
Immunosensor	Nanomaghemite/AuNPs/QD/peptide	bioconjugation	50pM	5-50nM	NR	NR	7.4	37	NR	NR	urine	Heger et al., 2015

LOD- limit of detection, V-voltage, MWCNT- multi wall carbon nanotubes, CuNPs- copper nanoparticles, AuE- gold electrode, μA- micro ampere, μM- micro molar, Mm- milli molar, s-seconds, pM- picomolar, Nm- nanomole, NR- not reported, Pt@ZIF8/GCE- platinum porous zeolitic imidazolate framework-8, SOxNPs- sarcosine oxidase nanoparticles, PVA-Ag/AuNPs-pphTEOS-SOD/GE- polyvinyl alcohol-silver and gold nanoparticles- partially prehydrolyzed tetraethyl orthosilicate- sarcosine oxidase- graphite electrode, p-AMTa/GCE -3-amino-5-mercapto-1,2,4-triazole on glassy carbon electrode, FMCA- ferrocene monocarboxylic acid, SOX/EDC/NHS/Au/ZnONPs/SPEs-sarcosine oxidase/N-ethyl-N-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide/gold- zinc oxide nanoparticles/screen printed electrodes, TCEP/MCH- Tris (2carboxyethyl)phosphine hydrochloride/ 6mercapto1hexanol, CuInS2/NiO/ITO-copper indium sulfide/nickel oxide /indium tin oxide, CdSe/ZnS/SOD/QD-

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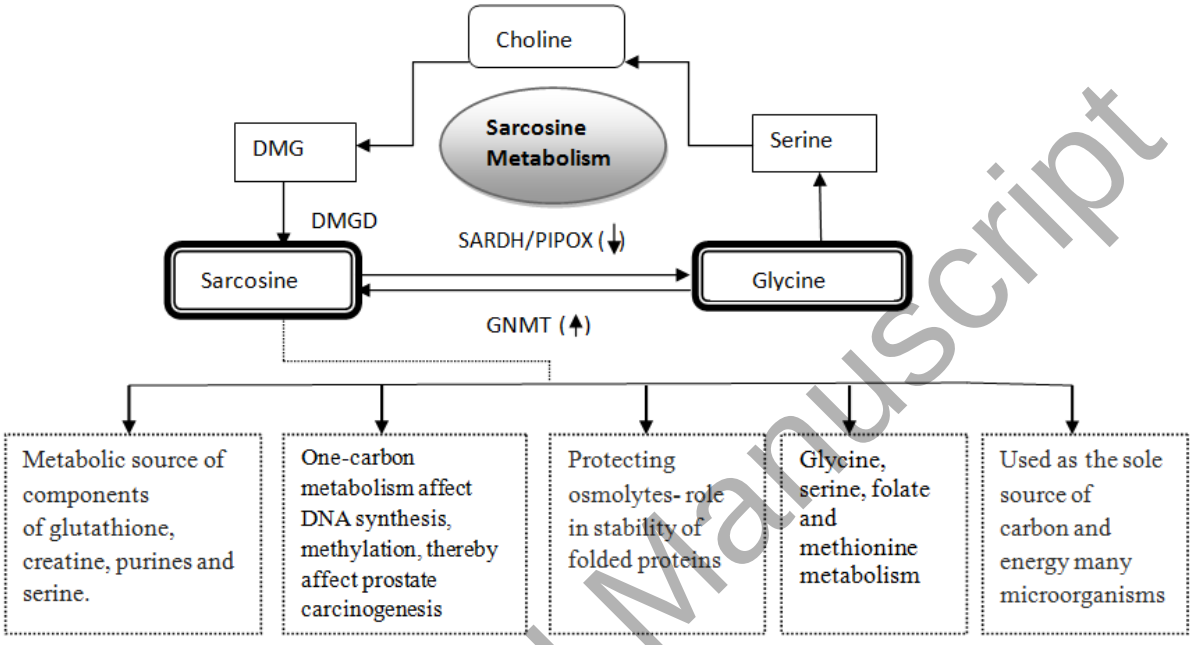
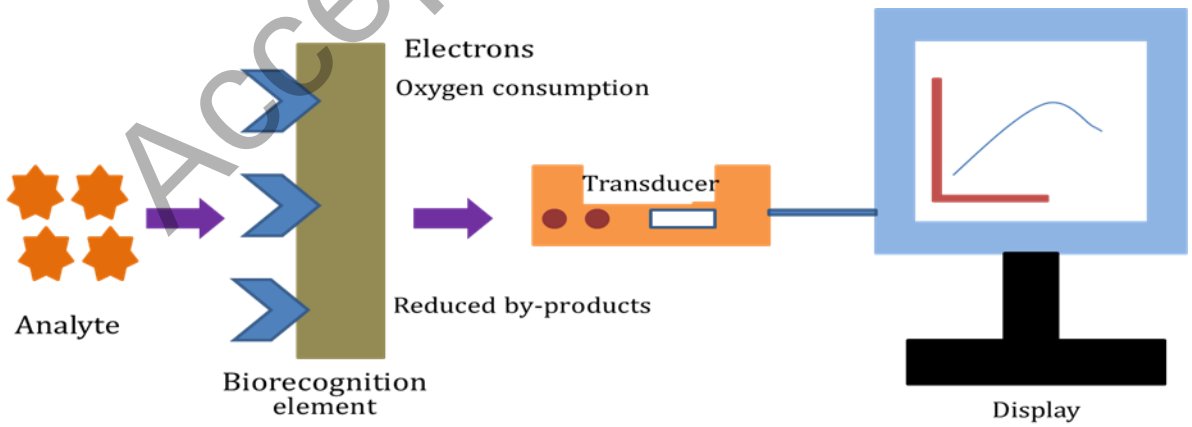
cadmium celenide/zinc sulphide/sarcosine oxidase ,AuNPs/QD- gold nanoparticles/quantum dots.





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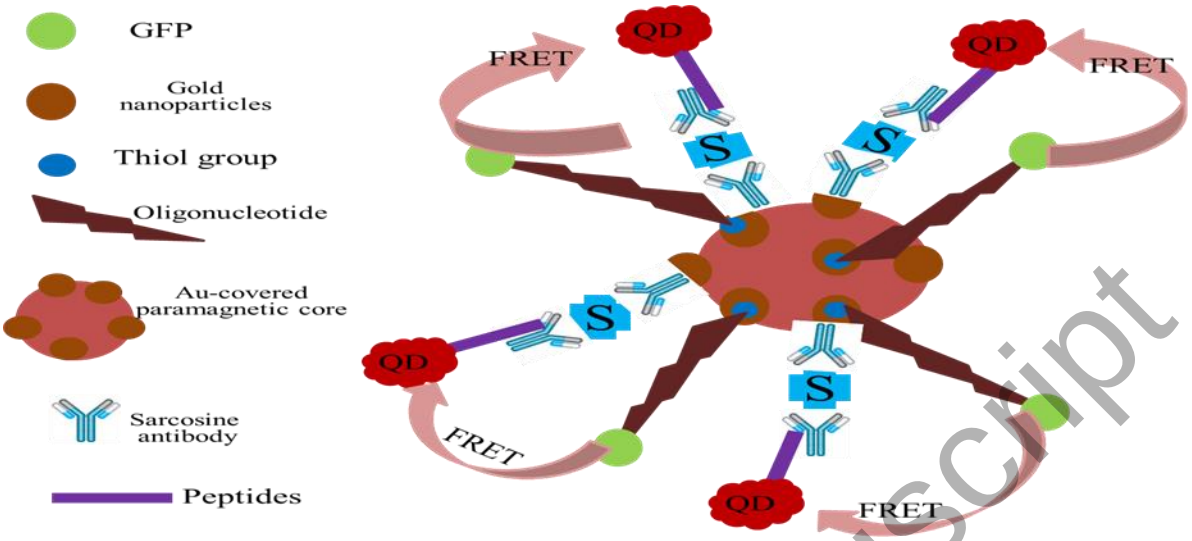

Figure caption

Figure 1: Overview of sarcosine metabolism and its potential physiological functions.

Figure 2: Diagram representing basic principle of biosensor working.

Figure 3: Expression of FRET between quantum dots (QDs) and green fluorescent proteins (GFP) and on surface of polyvinylpyrrolidone and gold modified paramagnetic nanoparticles

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