Int. J. Electrochem. Sci., 15 **(2020)** 7187 – 7202, doi: 10.20964/2020.08.04

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Review

Electroanalytical and Spectrophotometric Methods for the Determination of Melatonin-a Review

Hussain Alessa[1,*], *Jalal T. Althakafy*[1], *Amr L. Saber*[1,2]

[1] Department of Chemistry, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia
[2] Chemistry Department, Faculty of Science, Zagazig University, 44519-Zagazig, Egypt
[*]E-mail: hhessa@uqu.edu.sa

Received: 9 April 2020 / *Accepted:* 27 May 2020 / *Published:* 10 July 2020

Melatonin (MT), chemically N-acetyl-5-methoxytryptamine, was initially recognized in bovine pineal tissue and hence it has been represented exclusively as a hormone. The hormone can be used as a sleep aid for the treatment of some sleep disorders. However, MT is discovered in the earliest life forms and presented plants, bacteria, insects, fungi, and vertebrates as well as humans. MT possess various features that differentiate it from being a classical hormone, such as its direct, non-receptor-mediated free radical scavenging activity. Also, MT can be absorbed and consumed in foodstuffs such as wheat, vegetables, fruits, rice and herbal medicines. MT can also be classified as a vitamin. It seems likely that MT at first developed as an antioxidant, transformed to a vitamin in the food chain, and in multicellular organisms, where it is created, it has gained autacoid, paracoid and hormonal properties. This review concerns about different electroanalytical and spectrophotometric methods that have been used for the detection of melatonin, especially in the last three decades. It also illustrates the different electrodes materials and their modifications as they are the heart of sensors detection systems.

Keywords: Melatonin; Electroanalytical detection; Carbon-based electrodes; spectrophotometric detection.

1. INTRODUCTION

Melatonin (MT) is a short name for an organic compound derived from indole; N-acetyl-5-methoxytryptamine which was a result of pioneer work by Lerner et al [1]. This compound has a melting point in the range of 116 – 118 ^{o}C. It is a hormone synthesized in the pineal glands by pinealocytes [2,3]. This gland has a structure of a cone shape and is located in the brains of the animals. It is jointed to the roof of the third ventricle between the superior colliculi, immediately neighboring the habenular commissure [4]. Initially, tryptophan, an amino acid, flow in the bloodstream and is picked up from the bloodstream by the gland [5]. The tryptophan is said not to

affect the fabrication of MT as much as serotonin (SER), a precursor of MT, does [6]. The already mentioned precursor is catalyzed by tryptophan hydroxylase to tryptophan. Next, the same precursor can react with L-amino-decarboxylase to form 5-hydroxytryptamine, also known as SER. Arylalkylamine N-acetyltransferase (AANAT) can convert SER to L-acetyl-SER which determines the reaction rate. The production of MT is controlled by AANAT enzyme [7]. In human, AANAT is hosted on the 17q25 chromosome. The *o*-methylation of N-acetyl-5-hydroxytryptaime yielded MT, which has high affinity to water and lipids, hormone that through diffusion can enter and exit cells. MT is carried through the blood in an albumin-bound form, and 40 minutes is its half-life, thus it vanished fast. In livers, MT undergoes metabolism process to 6-hydroxyMT that combined to glucuronide or SO_4^{2-} and ejected to urine [6].

The bovine pineal tissues hosted the first discovery of tryptophan-derivative MT which was regarded as hormone. It was also identified in bacteria, insects, plants, algae and mammals. However, many evidences like its immediate, non-receptor-mediated free radical scavenging activity would alter its route as a hormone. Some regarded it as vitamin, from nutritional side, as it could be introduced to bodies via vegetables, fruits, herbs and rice. Thus, MT was said to have evolved as an antioxidant, being altered upon ingestion via food chain into vitamin and overall would has various properties [8].

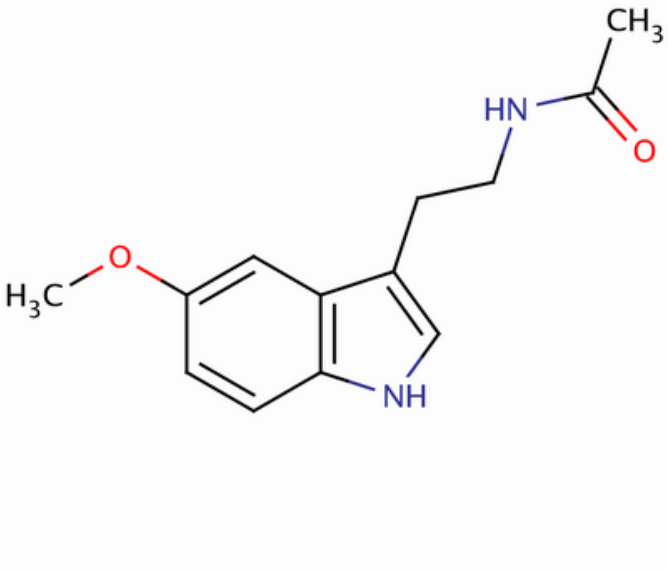

Figure 1. Structure of MT

The protective effect of MT on injuries was demonstrated by studying different concentrations of peroxynitrite anion (O=N-O-O). This anion is liberated from cultured neonatal myocardial cells, which is stimulated by ischemia/reperfusion. After fabricating the amperometry peroxynitrite ultramicro sensors, they underwent construction process. The process involved electropolymerizing inorganic macromolecular film of tetraaminophthalocyanine manganese (II), then chemically coated with poly(4-vinylpyridine). Peroxynitrite was determined with high selectivity and sensitivity, electrocatalytic reduction of peroxynitrite laid the detection process and the LOD was 0.018 μmol L^{-1}

(S/N of 3). UMS proved the direct in vivo measurement of peroxynitrite with no interference. Under optimum conditions, the UMS showed high "Peroxynitrite liberated from myocardial cells, both in the ischemic period and in the reperfusion period, was directly quantified. This approach leads to acquire some significant information about the role of myocardial cells on both the mechanism of injury and prospective treatments of medicine such as MT" [9].

MT and its derivatives found much useful use as chronobiologic regulators, e.g. physiological insomnia was treated by Neu-P11 and was shown to enhance sleeping in animals [10]. A study to see the effect of Neu-P11 on humans' plasma was conducted in which LC-MS/MS was used as a quantification method [10]. The samples were prepared by SPE. In HPLC, Purosper C18 was the stationary phase while the mobile phase was a gradient mixture of acetonitrile and ammonium acetate. The method proved its applicability to use on humans [10].

2. DETECTION METHODS OF MT

MT was detected in different samples, mostly biological ones. It was also detected in solid drugs, vegetables, fruits and rice samples. These samples were of either solid or liquid forms. Hence, based on the samples types, several procedures were used and developed for the preparation of the sample containing MT before the detection process. Among which are solid phase extraction (SPE) and liquid-liquid extraction (LLE).

Also, different analytical methods have been used for the determination of MT, these methods can be classified to chromatographic methods, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), tandem mass spectrometry coupled with ultra-performance liquid chromatography (UPLC/MS-MS). Also, spectrophotometric methods like second and third derivative spectrophotometry were used and electroanalytical methods. Chromatographic methods have shown potential use for MT analysis, which is supported by the highest number of publication in the literature. The work in this paper aims at summarizing the outcomes of the electroanalytical-based methods that were used for determining MT.

2.1 Electrochemical techniques

There are a variety of chromatographic techniques that have been used to determine MT in various samples types, however, these instruments are large, immobile and expensive to operate. Hence, a need for smaller, cost-effective, quick and easy to operate instruments is essential for the determination of MT. Electroanalytical methods possess such advantages. A summary of the electroanalytical techniques and the development of the sensors used for the detection of MT, including the electrode material (substrates), electroanalytical detection methods, analyzed samples and limit of detection are presented in table 1.

Table 1. Comparison of the performance of selected electroanalytical methods for melatonin sensing in different samples.

Electrode	Detection method	Analyte	Sample	Linear range (μg/L)	Detection limit (μg/L)	Year / Reference
CPE and GCE	LSV	Melatonin	Capsules	3.0 – 550	2.3	1991 / 11
CPE	Stripping voltammetry	Melatonin	Serum samples	0.05 – 0.1	0.001	1999 / 12
CPE		Melatonin	Urine samples	NA	NA	2000 / 13
CPE	CE-ED	Melatonin and pyridoxine	Commercial drug formulation	Melatonin (2.5 – 1000) Pyridoxine (5.0 – 1000)	Melatonin (1.3), pyridoxine (2.7)	2000 / 14
CPE	DPSV	Melatonin	-	2.0 to 10000	0.15	2000 / 15
GCE	CV and LSV	melatonin and pyridoxine	commercial tablets	Melatonin (20 – 80), pyridoxine (20 – 400)	Melatonin (5.86), pyridoxine (2.45)	2001 / 23
CPE	CV and FIA-ED	Melatonin	pharmaceuticals	CV (0.06 – 0.8) FID-ED (0.01 – 10)	CV (0.03) FID-ED (0.008)	2003 / 16
CPE	CV	Melatonin	Biological samples	NA	9×10^{-5}	2003 / 17
HMDE	SW-CASV	Melatonin	Human serum and pharmaceutical tablets		8.80×10^{-4} M (human serum)	2003 / 34
Bare MWCNT and modified MWCNT		Melatonin	Commercial capsules	0.08 – 10	0.02	2005 / 30
Bismus tetraiodate-drug ion-pair on a plasticized PVC membrane with o-nitrophenyl octyl ether or dioctyl phthalate	Potentiometry	Melatonin and oxomemazine	Urine and pharmaceutical formulations	Melatonin (1.0 – 10000) Oxomemazine (10.0 – 10000)	NA	2010 / 35
BDDE				80000 – 160000	0.06 mg/25 L	2012 / 31
BDDE	SWV	Melatonin	Urine samples and pharmaceutical	0.5 – 4.0	0.025 μg/ml	2012 / 32
Modified GCE	DPV and amperometry	Melatonin	Pharmaceutical product	0.5 – 14	0.019	2017 / 27
BDD	SWV	Melatonin and pyridoxine		11.0 – 260	0.82	2017 / 33
Modified CPE		Melatonin and tryptophan		0.02 – 6.0	Melatonin (0.0041) Tryptophan (0.0032)	2017 / 21
Modified CPE		Melatonin	Human serum and drug samples	0.06 – 140	21.6×10^{-3}	2019 / 22

Modified GCE	SWV	Melatonin, nicotine and norepinephrine	Commercial samples		Melatonin (2600) Norepinephrine (1400) Nicotine (1700)	2019 / 29
Modified gold electrode	SWV	Melatonin and serotonin	Biological samples	20 – 500	1.0	2018 / 36
Modified ITO electrode	CV and EIS	Melatonin	Biological samples	0.75 – 7.5	0.513 (CV), 0.175 (EIS)	2018 / 37
Modified GCE		Melatonin	Biological fluids	0.05 – 100	60×10^{-4}	2015 / 25
Modified GCE	CV and SWV	Melatonin	Drug tablets and urine	5 – 100	0.09	2016 / 26
Modified CPE	SWV	Melatonin and dopamine	Biological fluids and drug formulations	0.02 – 5.80	6.5×10^{-3}	2015 / 18
Modified GCE	DPV	Melatonin		0.05 – 13.5	0.0044	2018 / 28
Modified screen printed carbon electrode	CV and amperometry	Melatonin	Drug formulations		0.87	2016 / 20
Bare CPE, modified CPE with graphene, CPE modified with $CuCo_2O_4$	DPV	Melatonin	Organic samples	0.01 – 3.0 ($CuCo_2O_4$/CPE)	4.9×10^{-3} ($CuCo_2O_4$/CPE)	2015 / 19
GPT/WPE	SWV	Melatonin	Biological and pharmaceutical samples	0.08 – 100	0.108	2020 / 39
Flexible paper electrode	Amperometry	Melatonin and ascorbic acid		Melatonin (2.3 – 2000) Ascorbic acid (0.04 – 2000)	Melatonin (0.7) Ascorbic acid (0.012)	2020 / 40
SnO_2/SnS_2@r-BSA2/SPE	SWV	Melatonin	Biological samples	0.2 – 1000	0.016	2020 / 41

NA, not applicable; CPE, carbon paste electrode; GCE, Glassy carbon electrode; BDDE, Boron-doped diamond electrode; LSV, Linear sweep voltammetry; CE-ED, Capillary electrophoresis coupled with electrochemical detector; DPSV, Differential pulse stripping voltammetry; CV, Cyclic voltammetry; SWV, Square-wave voltammetry; FID-ED, amperometric detection in a flow injection analysis; WPE, waterproof paper electrode.

Various electroanalytical methods were used for the detection of MT. Selected ones are shown in table 1. These methods utilized various types of electrodes for MT study, among which the electrodes that used carbon as backbone gained extreme interest in studying MT. This is due to their advantages, i.e. cost-effective, conductivity, reliability, disposability and portability.

2.1.1. Carbon electrodes

A variety of carbon-based electrodes have received a special attention in the determination of MT, as can be seen in table 1. These electrodes can be carbon paste electrode (CPE), glassy carbon electrode (GCE), multi walled carbon nanotubes (MWCNT) and doped diamond electrode (DDE).

2.1.1.1. Carbon paste electrodes (CPE)

Electrochemical oxidation of MT, in capsules, in aqueous buffered solution, was performed by using carbon paste electrode (CPE) and glassy carbon electrode (GCE) [11]. The characteristics of this process were proven to be irreversible, regulated by diffusion and adsorption nature at the two electrodes during the whole ranges of 1.0 – 12.0 pH (11). MT was determined quantitatively at the CPE by voltammetry measurement in 20 mM perchloric acid. A calibration curve, which shows the relation between varied concentration and the peak current, obtained by linear sweep voltammetry (LSV) scan was linear in the area of 3.0×10^{-6} – 5.5×10^{-4} M and the LOD was 2.3×10^{-6} M (11). They stated that the nature of the used electrodes influence the resulted oxidation peak shape and position on the voltammogram. At GCE, broader peaks were observed with potential more positive by 0.03 V compared to CPE [11]. The closeness of the positions of the oxidation peaks of other indolic compounds made the simultaneous detection of MT difficult [11].

In addition, CPE was used for the pre-concentration of MT in which castor oil was added to CPE to extract MT from serum samples [12]. Stripping voltammetry of the extracted MT was evaluated taking into consideration other reaction variables such as, pH values and pre-concentration time [12]. A linearity in the calibration curve was noticed within the ranges of 0.05 – 0.1 μmol L^{-1} with 1.0×10^{-3} μmol L^{-1} as the LOD [12].

Another research was conducted for determining MT, in urine specimen, at two types of CPE; modified and non-modified ones. Both electrodes experienced a linearity response between MT and the peak current [13]. Possible interference from sample solutions was eliminated by using the open circuit pre-concentration/medium exchange/voltammetric scheme [13].

Furthermore, CE-ED was employed for the determination of MT and pyridoxine in medicinal drugs [14]. 12 minutes were enough for the separation and detection of both compounds. The sensing electrode was a 500 μm diameter carbon electrode, with an operation configuration of a wall-jet. The calibration curve shows linearity within 2.5 – 1000 μmol L^{-1} and 5 – 1000 μmol L^{-1} concentration ranges for MT and pyridoxine, respectively (14). LODs were 2.7 μmol L^{-1} and 1.3 μmol L^{-1} for pyridoxine and MT, respectively [14].

In addition, the adsorptive/extractive behavior of MT at CPE was explored. The adsorption and extraction were practically demonstrated, showing that they are both of prime roles during pre-concentration process, considering adsorption having lower importance compared to the extraction. Differential pulse stripping voltammetry (DPSV) was employed for the determination of MT, this was done at CPE. This was performed against Ag/AgCl electrode, and it showed a sensitive anodic peak at 0.7 V. Moreover, a linear response was noticed within the concentration ranges of 2.0 to 10000 μmol

L^{-1} and the detection limit was 0.15 μmol L^{-1}. The sensitivity, simplicity and the rapid method was demonstrated [15].

MT in pharmaceuticals was detected in flow injection analysis (FIA) systems by CV and amperometry [16]. Very good reproducibility was obtained via two processes; careful pretreatment of the CPE and applying a high flow rate. Ten and twenty samples were analyzed; the obtained RSD values were 1.5% for the ten samples whereas 1.3% was registered for the twenty ones. Although the LODs were low, a linear dynamic range (LDR) trifold of magnitude, 0.01 – 10 μmol L^{-1} was obtained when coupling with FIA. The two methods proved themselves in analyzing MT in pharmaceutical samples, even when fluorescent procedure was employed. The coupling methods were fast, cost-effective, easily automated and simple to use [16]. It is reported that applying high voltages, 1.1 to 1.6 V, for a minute and less would remove the adsorptive products and renew the electrode surface [16].

Costa-Garcia et al carefully studied the parameters that influence the signals of CV of MT. This was conducted on biological samples to determine MT using voltammetric measurements. After using the optimized alternating current voltammetry (ACV), a LOD of 9×10^{-5} μmol L^{-1} was acquired. A pretreatment of the CPE, at which the redox process of MT take place, led to good reproducibility, with relative standard deviation of 2.7% for 10 samples [17].

Bagheri et al modified CPE for the detection of MT and dopamine in biological fluids and drug formulations. They modified the surface of CPE with reduced graphene that had been decorated with Fe_3O_4 nanoparticles. When using SWV, the analysis of both analytes resulted in a linear calibration curve in the ranges 0.02 – 5.80 μmol L^{-1}. LODs were 6.5×10^{-3} μmol L^{-1} and 8.4×10^{-3} μmol L^{-1} for MT and dopamine, respectively [18].

Tadayon and Sepehri systematically studied the detection of MT, in several organic samples, using pristine CPE, graphene-modified CPE, $CuCo_2O_4$/CPE as well as treating the latter electrode with nitrogen-doped RGO. MT was detected simultaneously in the presence of dopamine and tryptophan. DPV when using the latter electrode revealed a linearity range of 0.01 – 3.0 μM for all the analytes with LOD of 0.0049 μM for MT [19].

Additionally, MT was detected in drug formulations by CV and amperometry. Apetrei used graphene-modified screen-printed carbon electrodes for this process. The use of graphene enhanced the electroactive sites leading to improving the sensitivity of the carbon electrode. They reported LOD of 0.87 μM [20].

Bagheri et al developed a new sensor for concurrent determination of MT and tryptophan. The sensor was made by modifying CPE with reduced graphene oxide then decorated with SnO_2-Co_3O_4. The proposed electrode exhibited a linear response in the ranges 0.02 – 6.0 μmol L^{-1} with a detection limits of 0.0041 μmol L^{-1} and 0.0032 μmol L^{-1} for MT and tryptophan, respectively [21].

A sensor utilized Al_2O_3-supported Pd nanoparticles for modifying CPE was used for determining MT. The treatment of CPE with these nanoparticles enhanced the detection limit and the sensitivity of the used voltametric methods. This improvement stems from the electrocatalytic behavior of the nanomaterials which enhanced the surfaced area of the CPE and increased the electrode's charge transfer. Authors reported the application of the method on human serum and drug specimen, which resulted in linear calibration curve in 0.06 – 140 μmol L^{-1} concentration range with a LOD of 0.021 μmol L^{-1} [22].

2.1.1.2. Glassy carbon electrodes (GCE)

Differential pulse voltammetry (DPV) with GCE was employed for simultaneous determination of MT and pyridoxine. In this method, the drugs underwent oxidation process at the carbon electrode. The parameters influencing the oxidation process, such as the nature of the buffer, pH, the concentration and the scan rate were all studied by CV and LSV. The method was developed, by using 5×10^{-2} M sulfuric acid with 20% methanol, which allowed simultaneous determination of MT and pyridoxine hydrochloride even if they present together. Linear ranges were reported as 20 – 80 0.06 – 140 μmol L^{-1} and 20 – 400 0.06 – 140 μmol L^{-1} with LOD of 5.86 μM and 2.45 μM, for MT and pyridoxine, respectively. The method experienced, with no interference, successful application on commercial tablets [23].

A report used DPV to study the oxidation processes of MT and vitamin B6 at GCE. It concluded that oxidation anodic peaks were observed at 0.65 V, versus silver/silver chloride electrode in ammonia buffer solution at pH=9, and at 0.84 V, versus same reference electrode in Britton-Robison buffer solution at pH=2, for vitamin B6 and MT, respectively. Moreover, quantification was proved via the linear relation between the peaks current and the concentrations [24]. Goyal and Gupta fabricated a modified GCE for the detection of MT in biological fluids. MIP, molecularly imprinted polymer, sensor was made by depositing graphene layer on GCE before electropolymerzing AHNSA and melamine (MM). A linear relationship between the oxidation peak current and MT concentration was obtained within 0.05 – 100 μmol L^{-1}. LODs was 60×10^{-4} μmol L^{-1} [25].

An advancement was made by modifying the surface of GCE by electro-polymerization of AHNSA; 4-amino-3-hydroxy-1-naphthalenesulfonic acid, then treated with graphene oxide and palladium particles. These modified GCE were used to determine MT in solid-formulation drugs and urine samples by using CV and SWV. Linearity was noticed in 5 – 100 μmol $L^{-1}$with LOD of 0.09 μmol L^{-1} [26]. Further modification of GCE was made by carbon black and employed DPV as well as amperometry for the determination of MT in pharmaceutical products. A LOD of 0.019 μg L^{-1} was obtained. In using amperometry, MT showed a linearity in the calibration curve within the range 0.5 – 14 μmol L^{-1} [27].

A new composite for the modification of GCE was fabricated and employed DPV for the quantification of MT. This was done by employing electrocatalytic agents of nanoparticles of Fe_3O_4, which had been decorated with carbon quantum dots, for detecting MT. This resulted in LOD of 0.0044 μmol L^{-1} and the linearity in the concentration range 0.05 – 13.5 μmol L^{-1} [28]. Moreover, MT was simultaneously detected, along with nicotine and norepinephrine in commercial samples. The determination was done by using a biosensor of NAD/GI EDTA-WO_3/GCE using SWV. (NAD = nicotinamide adenine dinucleotide LODs were 2.6×10^{-3} μmol L^{-1} for MT, 1.4×10^{-3} μmol L^{-1} for norepinephrine while 1.7×10^{-3} μmol L^{-1} for nicotine [29].

2.1.1.3. Multi Walled Carbon nanotubes (MWCNT)

Other type of carbon electrodes were used for the EC study of MT behavior. Bare Multi-wall carbon nanotubes (MWCNT) and MWCNT which had been coated with GCE were used for the study

[30]. An increase in the MT's oxidation peak current was observed when using the coated MWCNT, compared to the bare GCE, and a positive shift of the peak position. This comparison proved the feasibility of using MWCNT for the EC oxidation of MT. The method was applied on commercial capsules, and when changing the concentration, a proportional relation with a linear response from the oxidation peak current, 0.08 – 10 μM is demonstrated with 0.02 μM as the LOD [30].

2.1.1.4. Doped Diamond electrodes (DDE)

In addition, a rapid analysis, within 2.5 minutes, of MT tablets was reported by Patel et al. A BBDE was employed as the sensing electrode whereas the reference electrode was Ag/AgCl. The oxidation of MT was at +0.8 V with respect to the reference electrode. No extensive sample preparation was necessary prior to the voltammetric measurements. Yet, the EC response was affected by the presence of carboxymethyl cellulose and dicalcium phosphate. A linear response was observed over a range of 80000 – 160000 μmol L^{-1} of MT, and 2400 μmol L^{-1} was obtained as the LOD [31]. Additional positive feature of this method is that use of ethanol-wet cloth would refresh the surface of the working electrode [31].

Square-wave voltammetry (SWV) was used by Levent to quantify MT electro-analytically. The analysis was done on urine samples and pharmaceutical ones. BBDE was employed as the sensing electrode while Ag/AgCl was used as the reference electrode, and Britton-buffer solution at pH = 3.0. Clear voltammetric oxidation peaks were observed at 0.88V versus the reference electrode. From the peaks, MT was determined in the linear range of 0.5 to 4.0 μmol L^{-1} (r = 0.998, 8 samples) with 0.025 μg ml^{-1} as the LOD. 2.06% was reported as relative standard deviation at 2.0 μg L^{-1} (for ten samples). 97.67 – 105% were reported recoveries of MT in tablet and spiked human urine samples [32].

A cathodically pretreated BDD electrode was used during the simultaneous determination of MT and pyridoxine. This treatment enhanced the measurement repeatability and the current sensitivity. SWV study revealed a linear calibration curve within the ranges of 11 – 260 μmol L^{-1} with 0.82 μmol L^{-1} as LOD of MT and 2.7 μmol L^{-1} as LOQ of MT [33].

2.1.2. Other electrodes

Other electroanalytical technique like square-wave cathodic adsorptive stripping voltammetry (SW-CASV) was used for determining MT in human serum as well as pharmaceutical tablets. This was done by reducing the hormone on a Hanging mercury drop electrode (HMDE), which was followed by studying the reduction processes using two buffers; acetate (pH = 4.5-5.5) and Britton-Robinson (pH = 2-11).Careful study outlined the acetate buffer with pH =5.0 is the optimum electrolyte for the MT analysis. When using -0.65 V as the accumulation potential, the method registered a single-wall peak at -1.45V against Ag/AgCl/KCl. The functional group in the reactant; the double bond in C=O, may have caused the appearance of the peak [34]. Also, MT hormone has a crucial role in many discrete physiological functions [34]. A mean recovery for 0.01 μmol L^{-1} M MT in bulk form followed 30 s accumulation of 98.87 ± 0.78 % and a LOD of 3.13×10^{-4} μmol L^{-1} were

achieved. Conducting the procedure on tablets and human serum gave 97.68 ± 0.57 % and 101.67 ± 0.85 % as the mean recoveries for the already mentioned sample's type, respectively. When applied to human serum, the LOD of the MT was 8.80×10^{-4} μmol L^{-1}. It should be mentioned that, there was no interference from other compounds, which are co-formulated with MT, like excipients and vitamin B6. Furthermore, other compounds such as 5-hydroxytryptophan, SER and tryptophan showed no significant interference effect [34].

MT and oxomemazine were detected in urine and pharmaceutical formulations by using modified sensors. The incorporation of bismus tetraiodate-drug ion-pair as electroactive materials with a plasticized Polyvinyl chloride (PVC) membrane with o-nitrophenyl octyl ether or dioctyl phthalate was used in potentiometric measurements. The modified sensors were conditioned in 0.1 M drug solution for 48 hours before use. A fast Nernstian response with high stability was obtained during the concentration range of 1 – 1.0×10^{4} μmol L^{-1} for MT whereas similar response was observed for oxomemazine in the concentration range 10 – 1.0×10^{4} μmol L^{-1}, the measurements were conducted for MT in pH = 3.0 – 6.5 whereas pH = 3.5 – 6.0 for oxomemazine. It should be mentioned that high selectivity was obtained by the modified sensors with no interference from other cations [35].

Also, simultaneous determination of MT along with SER was achieved by using a SWV performed at a modified gold electrode, in which the electrode was immobilized with acetylene black nanoparticles-chitosan. The determination was applied on biological samples and MT showed a linearity response in the ranges between 20 μmol L^{-1} and 500 μmol L^{-1} with a LOD of 1 μmol L^{-1} [36].

Brazaca et al successfully quantified MT in biological samples using CV and Electrochemical Impedance Spectroscopy (EIS) methods. MT was detected by a sensor having modified indium tin oxide electrode (ITO). (3-Aminopropyl)triethoxysilane (APTES), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) crosslinkers were used for immobilizing anti-MT antibodies on ITO electrode. A linearity was noticed within concentrations between 0.75 – 7.5 μg L^{-1} while 0.175 μg L^{-1} and 0.513 μg L^{-1} were obtained as LODs of MT when using EIS and CV, respectively [37]. Furthermore, a group of Chinese scientists developed a sensor for the detection of MT and pyridoxine in plants. The composite electrode; CuO-poly(L-lysine)/graphene, operates as its first part can detect the two active compounds while graphene enhance the sensitivity be amplifying the current. MT detection seen a linearity in 0.016 – 1110 μM with 0.012 μM as the LOD [38].

Owing to feasible features of waterproof paper as a sensor substrate, Janegitz et al. used it as a platform for constructing a sensor to determine MT and paracetamol in biological and pharmaceutical samples. MT and paracetamol were detected by using DPV and SWV, respectively. The application showed ability to detect MT as low as 0.032 μmol L^{-1} [39].

MT and ascorbic acid were simultaneously detected by using a flexible paper-based electrode. The electrode was fabricated by the chemical reduction of B_2O_3-graphene composition. Amperometrically, the electrode showed a linearity response in the ranges of 2.3 – 2000 μM and 0.04 – 2000 μM for MT and ascorbic acid, respectively. The detection limit was as low as 0.7 μM and 0.012 μM for MT and ascorbic acid, respectively. These good results were due to synergetic effect of the structure of the composite electrode [40].

SWV was used for detecting MT in biological samples. A composite of SnO_2/SnS_2@r-BSA2/SPE was used as the sensing electrode. The electrode showed a linear response in 0.2 – 1000 μM range with 0.016 μM LOD. It was proven that the MT was detected even under high concentration of the interfering compounds [41]. Very recently, it was shown that no electroactive interferences were noticed during the analysis of MT using different electrodes; GCE, Au and Pt based electrodes in aqueous solutions [42].

2.2 Spectrophotometric techniques

The literature has comparatively few spectrophotometric methods, standing by their own and not coupled to chromatographic methods, which have been used to determine indole-based compounds. One of which was a research developing a spectrophotometric method for quantifying indoles that exist in nature [43]. The method had good precision, simple to use and quick in performing the analysis. A 2nd and 3rd derivative spectroscopy was used for simultaneous determination of MT and pyridoxine in medicinal tablets. This method was conducted in 0.1 M HCl and gave LODs of 50 and 260 μmol L^{-1} for MT and pyridoxine, respectively, at 95% confidence level [43]. Moreover, combined MT and pyridoxine HCl were investigated in commercial tablets and laboratory-made ones. The analysis was made by means of two methods. As for the first one, it employed 1st and 2nd derivatives UV with zero-crossing technique. The basis of the second method was to rely on the native fluorescence of MT and pyridoxine hydrochloride. Both methods gave satisfactory results [44].

Single MT tablets and MT-pyridoxine tablets were analyzed and no interference was observed. When using colorimetrical method, combined tablets produced higher recoveries, > 100%, than single MT tablets, ≤ 100%, with detection limit of 270 μmol L^{-1}. The spectro-fluorimetrical method, recoveries were 100.2 + 0.39 and 100.61 for combined and single tablets, respectively, with 0.35 μmol L^{-1} as the LOD [44]. Tablets containing MT were subjected to photo-stability study in which their degradation at various pH values was detected by HPLC. The extracted MT was then studied by NMR, FT-IR and MS [45].

Table 2. Comparison of the performance of selected spectrophotometric methods that have been used for the determination of melatonin in different samples.

Detection method	Analyte	Sample	Linear range (μg/L)	Detection limit (μg/L)	Year / Reference
2nd and 3rd derivative spectroscopy	Melatonin and pyridoxine	Pharmaceutical tablets	N.A.	Melatonin (50) Pyridoxine (260)	1998 / 43
Colorimetry and Spectro-fluorimetry	Melatonin	Pharmaceutical tablets	Colorimetry (1250 – 10000) Specrofluorimetry (20 – 80)	Colorimetry (270) Specrofluorimetry (0.35)	1998 / 44
Colorimetry	Melatonin	Pharmaceutical formulation	Method 1 (0.4 – 6.4) Method 2 (0.4 – 7.4)	N.A.	1999 / 46
Chemiluminescence	Melatonin	Pharmaceutical	0.25 – 250	0.1	2001 / 48

		tablets			
Colorimetry	Melatonin	Pharmaceutical formulation	Method A (800 – 14200) Method B (70000 – 140000) Method C (2000 – 40000)	Method A (80) Method B (60) Method C (150)	2000 / 49
Spectro-fluorimetry	Melatonin	Pharmaceutical and biological samples	50 – 3000	10	2000 / 50
1 st derivative spectroscopy	Melatonin and pyridoxine HCl	Pharmaceutical samples	Melatonin (1000 – 30000) Pyridoxine (1000 – 22000)	Melatonin (240) Pyridoxine (280)	2002 / 52
Fluorescence	Melatonin	Pharmaceutical samples	100 – 1000	25	2003 / 54
HAI-RTP	Melatonin	Pharmaceutical samples	10 – 200	3.6	2005 / 57
Chemiluminescence	Melatonin	Biological and medicinal samples	0.1 – 250	0.05	2006 / 58

NA, not applicable; HAI-RTP, heavy atom-induced room temperature phosphorimetric

MT was detected by two simple methods based on forming colored complexes. One by the reaction of MT with iron(III)-o-phenanthroline (method 1), the other is by reducing iron (III) (method 2). Then measuring the absorbance at λ_{max} of 510 nm and 522 nm for the methods 1 and 2, respectively. The linear line, concentration versus the absorbance, was observed within 0.4 – 6.4 μmol mL^{-1} and 0.4 – 7.4 μmol mL^{-1} for methods 1 and 2, respectively. It was stated that raising the temperature up to 70 ^{o}C would accelerate the complex color development and shorten then analysis time, when compared to the room temperature [46]. Also, a study to detect MT in medicinal tablets was conducted by using 1st derivative UV [47]. The method had a calibration curve with linear ranges of 15 – 45 ppm in pH ranges of 5 – 9. No interferences were noticed during the measurements. Upon varying the methods' parameters, the temperature of sonication process was the most influential parameters for the successful MT detection. The study stated that accurate spectrophotometric determination was obtained when the samples were completely dissolved, which was at < 45 ^{o}C [47]. MT was detected by chemiluminescence method in which the weak chemiluminescence between hydrogen peroxide and acetonitrile would be increased by the presence of MT. 0.25 – 250 μmol mL^{-1} was the log-log linear range with the LOD of 0.1 μmol mL^{-1}. The MT chemiluminescence could have been resulted from the singlet oxygen that was yielded from the reaction of acetonitrile and hydrogen peroxide [48].

Three colorimetric methods were used for the estimation of MT in pharmaceutical formulations. This is by the reaction of the object with a reagent to form a colored compound. The individual reagents were ninhydrin (A), $K_2S_2O_8$ (B) and OsO_4 (C). The absorbance of the colored compound was measured at 397 nm, 450 nm and 516 nm for A, B and C methods, respectively. It was observed that the stability of the colored species followed the order of A > C > B. The linearity was in the ranges of 0.8 – 14.2 μg mL^{-1}, 70 – 140 μg mL^{-1} and 2 – 40 μg $mL^{-1}$for A, B and C mthods, respectively. The methods yielded recoveries up to 100% [49]. The fluorescent emission spectra of the

interaction of MT with different cyclodextrins (α, β and γ CDs) to form complexes were studied. This was applied for the detection of MT in pharmaceutical and biological samples. It was noted that the acidity did affect the emission and the reaction with 10 mM methyl-β-cyclodextrin gave detection limit of 10 μmol L^{-1} with 50 – 3000 μmol L^{-1} linearity range [50]. The stability, solubility of these complexes MT with different CDs (α, β and γ) was studied by NMR. The stability in aqueous mediums was ordered as α-CD < γ-CD < β-CD [51].

MT and pyridoxine hydrochloride were detected in the presence of each other by spectrophotometric method in which the ratio spectra of the 1 st derivative was used [52]. The analysis was done on synthetic mixtures and medicinal formulations. To obtain this ratio, a standard spectrum of one of the compounds should be made, then the absorption spectrum of their mixture should be made, the division of the latter by the former would give the ratio spectra. The establishment of Beer's law was seen in the calibration graphs within the concentration ranges of 1000 – 30000 μmol L^{-1} for MT whereas 1000 – 22000 μmol L^{-1} for pyridoxine [52]. MT and its derivatives were found to produce chemiluminescence in acidic solution of $KMnO_4$ whereas the chemiluminescence reaction would be increased massively by formaldehyde [53]. MT was extracted from pharmaceutical tablets by liquid-liquid extraction and analyzed by studying the effect of solution concentration on the fluorescence emission. The linearity was seen in 100 – 1000 μmol L^{-1} concentration range with 25 μmol L^{-1} as the LOD [54].

MT, its precursors and its metabolites were detected by CE with either UV detector or fluorometric detector [55]. The studied compounds were MT, 6-HMT, SER, tryptophan, 5-methoxytryptamine and 5-methoxyindoleacetic acid. A mixture of a buffered 0.02 M borate solution with 0.05 M sodium dodecyl sulfate was used as electrolyte for their separation and they were separated within less than 15 minutes at lower than ppm values [55]. The monitoring of MT in pharmaceutical solid drugs was done by CE-UV which gave a relative standard deviation of 1.6%. MT was extracted from biological samples, e.g. plasma samples, with either ethyl acetate or chloroform. 82.1% and 87.2% were reported as the recoveries of MT when extracted by ethyl acetate and chloroform, respectively. It concluded that the use of fluorometric detector gave better results than using UV [55]. MT and pyridoxine were spectrofluorimetrically and spectrophotometrically simultaneously detected in pharmaceutical preparations by using partial least squares calibration and principle component regression methods, shorten to PLS and PCR, respectively [56]. As no direct determination of these two compounds could be used by either spectro methods due to the overlap of their absorption and emission spectra, the need to develop full-scan calibration by PLS and PCR was a necessity. A mixture of the two drugs was prepared followed by measuring the fluorescence emission, by fluorimetry, in wavelength ranges 324 – 500 nm with excitation wavelength of 285 nm. Then recording the absorption spectrum within 250 – 350 nm with λ_{max} of 310 nm or of 278 nm for pyridoxine and MT, respectively [56].

The phosphorescence properties of MT were studied, in commercial drug preparations, by heavy atom-induced room temperature phosphorimetric (HAI-RTP) methods [57]. The RTP signal was produced by the reaction between heavy atom sources, like KI, was mixed at 1.2 M with 2 mM Na_2SO_3, which acted as deoxygenating agent. 290 nm and 457 nm were the wavelength at which MT produced the highest phosphorescence emission and excitation, respectively. In aqueous solutions, MT

could be detected in 10 – 200 μmol L^{-1} with LOD of 3.6 μmol L^{-1} [57]. Another application in chemiluminescence method to detect MT was published [58]. In this research, MT was reacted with both H_2O_2 and NaOCl in alakaline medium to produce chemiluminescence emission by the reaction product; singlet oxygen. The analysis was performed on rat pineal glands and medicines. A concentration calibration curve was linear in the ranges of 0.1 – 250 μM with 0.05 μM as the LOD. No significant interference was observed by glucose, cations, anions, starch or indole-based compounds [58]. Additionally, another use of spectrofluorimetric and spectrophotometric method for the determination of MT is described. Within the articles, a development in the so-called "net analyte signal standard addition method" which is shorten to NASSAM standard method was discussed [59]. Analytes like MT and pyridoxine were determined under the influence of some interfering compounds. The NASSAM was coupled with the spectro methods to determine the analytes of interest, in fabricated mixtures and in drugs formulations, with no significant interference from other compounds [59].

The presence of its main contaminant, N-{2-[1-({3-[2-(acetylamino)ethyl]-5-methoxy-1*H*-indol-2-yl}methyl)-5-methoxy-1*H*-indol-3-yl]ethyl}acetamide (DMLT) could affect the accurate reading of MT concentration. Thus, a new spectrofluorimetric method was proposed for solving this matter [60]. To do so, this compound was laboratory-made synthesized from 5-methoxyindole-2-carboxylic acid, and MT was determined in laboratory-made mixtures and in commercial tablets. It was proved that this contaminant had no significant influence on MT determination as long as its concentration is 60% and less. MT was detected and its recoveries were no less than 99% [60]. Another spectrofluorimetric method was developed for the detection of MT even when its contaminant, DMLT, is present. The developed method was simple, cost-effective, rapid, low chemical consuming and has high sensitivity [61]. The same group further developed a method for spectrofluorometric detection of MT in laboratory-made samples and pharmaceutical drugs. The development was based on 4 different calibration models. Recoveries up to 99% were obtained for MT with no noticed effect from the interfering contaminant, DMLT, neither pyridoxine HCl [62]. A systematic study by Mexican researchers proved that the surrounding environment, like the light and oxygen concentration, has impacted the stability of MT solution, hence its pKa. MT stability increased by removing the effect of the surrounding, which resulted in calculating its acidity constants to be 5.772 ± 0.011 and 10.201 ± 0.024 [63].

3. CONCLUSION

In this review, different types of electroanalytical and spectrophotometric techniques for the detection of melatonin in various samples have been covered. It is obvious that electroanalytical methods dominate the detection arena with various methods and lower detection limits. However, high-performance liquid chromatography with electrochemical or with fluorometric detection systems is the most popular as an analytical tool for the determination of MT and other indole related compounds.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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