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Review—Electrochemical Sensors Used in the Determination of Riboflavin

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Riboﬂavin or vitamin B12 (Fig. 1) is a B group water soluble vitamin and an essential constituent of ﬂavoenzymes which plays a vital role in the biochemical reactions in the human body. All human and animal beings need a constant supply of riboﬂavin. Its deﬁciency is associated with eye lesions and skin disorders. Riboﬂavin cannot be formed in the human body, thus has to be obtained from food (such as liver, cheese, fruit and vegetables) and pharmaceutical products. Several analytical methods are used for the determination of riboﬂavin, such as chemiluminescence (CL), optical techniques based on measuring the intensity of ﬂuorescence (FL), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). In general, ﬂavins can be detected and determined using different spectrometric methods. These methods provide high sensitivity, but in comparison with electrochemical techniques, all the above mentioned techniques are complicated, time consuming, and expensive. Electrochemical methods are more simple, sensitive, low cost and they show rapid response time. The electrochemical methods which are widely used are potentiometry, cyclic voltammetry, differential pulse voltammetry, square wave voltammetry and chronamperometry. The design of the electrochemical sensors is simple and it requires a small amount of material. Due to these advantages, a series of electrochemical methods for the determination and detection of riboﬂavin using different types of electrodes were proposed.

An important tool in the improving of the performance of the electrode is the addition of a chemical modiﬁer or the functionalization of the material base of the electrochemical sensors.

The determination of riboﬂavin can be performed from different matrices such as: pharmaceutical products, cow and powdered milk, non-alcoholic beer or human plasma. This review is mainly focused on the electrochemical sensors applied in real pharmaceutical samples. Due to the simple preparation of the samples it was favorable to investigate the applicability of the proposed sensors in multivitamins capsules, tablets and nutrient products. Pharmaceutical products possess a simple matrix compared to the biological samples and the working electrodes exhibit a series of advantages such as sensitivity, stability, selectivity, reproducibility and freedom from interference.

One of the most sensitive and selective type of sensors are the biosensors, but they require a qualiﬁed personal for their preparation and manipulation. Even if they show high sensitivity in the analyte determination, the biosensors are disposable which involves high costs for the materials and speciﬁc storage parameters. Further a credit will be given for the simplest preparation procedure of the electrochemical sensors (such as glassy carbon and carbon paste electrode) and their applicability in the determination of riboﬂavin.

**Electrochemical Sensors for Detection of Riboﬂavin**

The electrochemical sensors are based on different types of materials which possessed conductive properties linked with the electron transfer. In the riboﬂavin’s reduction process two electrons and two protons are involved (Scheme 1). In the past few years, the electrochemical determination of riboﬂavin was realized using sensors manufactured from graphite, glassy carbon, graphene, carbon nanotubes, diamond paste, materials based on metallic nanoparticles such as gold-, silver-nanoparticles, and so on. Metal based electrodes (gold and platinum) possessed a series of disadvantages in the electroanalytical applications such as: limited in the positive potential range because the surface is getting oxidized and the same process occurs in the negative range and the potential overload of the electrode surface with impurities it is high. Numerous reports are available on the electrochemical detection of riboﬂavin on different modiﬁed electrodes made up of metal oxides and their composites with other metal oxides, carbon materials or polymers. The sensing efﬁciency of these materials towards riboﬂavin has been improved by using different approaches like tuning their structural, morphological, physical, chemical or catalytic properties. The technological progress in electrode preparation techniques also plays a major role in order to bring the novel ideas into real time applications. The porous nanomaterials were widely used due to their high surface area compared to the bulk, which can provide high accessible active sites to interact with probe molecule in sensors’ applications.

This review has been focused on the modiﬁed glassy carbon and carbon paste electrodes developed in the last few years for the determination of riboﬂavin.

**Carbon paste electrode (CPE) used for determination of riboﬂavin.**—The carbon paste electrodes (CPE) and chemically modified carbon paste electrodes are widely applied in several ﬁelds of electrochemistry. The CPEs are cheap to manufacture, they present easy ways of preparations, and their active surface area can be fast renewed. They also exhibit reproducible surface area and low residual current in wide potential windows. In generally the preparation method of the CPE is described by Adams in 1958, presented as a mixture between a carbon material and an organic liquid (bromoform) inserted in a Teflon rod, whilst the electrical contact was provided by a platinum wire. CPE is widely used as a working electrode caused by the simplest procedure to modify the material. Other series of advantages are uniform distribution of the catalyst/modiﬁer into the paste, very low Ohmic resistance, a better stability and robustness in aqueous solutions.
Nezamzadeh-Ejhieh and Pouladsaz proposed a CPE modified with Co\textsuperscript{2+} Y zeolite used in voltammetric determination of riboflavin. By using Co\textsuperscript{2+} Y zeolite in the matrix of the sensors a good selectivity was provided based on the size (and shape) of the reactants,\textsuperscript{29} together with a three dimensional lattice made of interconnected cages of molecular dimension coming with a variety of support sites for various catalysts. The manufacture method used in the preparation of the sensors follow the classical pattern by mixing an appropriate amount of Co\textsuperscript{3+} zeolite with graphite powder and in the end Nujol (mineral oil which substitute the paraffin oil) was added. According with this research, the sensibility was significantly in influence the electro-oxidation of riboflavin. The results obtained for the determination of riboflavin in pharmaceutical products samples, by using the developed method, were in agreement with those obtained by the American Organization of Analytical Chemists (AOAC) method.\textsuperscript{30}

Mehmeti and collaborators developed a simple and cheap procedure for the determination of riboflavin at low concentrations based on a manganese dioxide bulk-modified CPE (MnO\textsubscript{2}/CPE). MnO\textsubscript{2} based electrodes are very popular due high catalytic effect at an appropriate potential for sensing the target analyte.\textsuperscript{31} The dynamic concentration range was from 0.02 to 9.00 \( \mu \text{mol l}^{-1} \), the detection limit (3\( \sigma \)c=0.006\( \mu \text{M/slope} \)) was estimated to be 15.00 \( \text{nmol l}^{-1} \). The method has a good selectivity for the electrochemical determination of riboflavin and the recovery rate of riboflavin from pharmaceutical formulations was higher than 95.00\%. The repeatability (n = 4 measurements, C = 0.06 \( \mu \text{mol l}^{-1} \) riboflavin) was calculated to be \( \pm 2.00\% \). The RSD, and the reproducibility of the electrode preparation, based on measurements of the 0.06 \( \mu \text{mol l}^{-1} \) of riboflavin with four separately prepared electrodes, was estimated to be \( \pm 3.00\% \), which proved that this electrode can be a satisfactory replacement for commercial electrodes.\textsuperscript{32}

The group of Khaloo introduced a new sensitive and selective electrochemical sensor for the determination of riboflavin, described by the incorporation of manganese (III) tetraphenyl porphyrine (MnTPP) as a modifier into carbon paste matrix. The electrochemical behavior of riboflavin on the surface of modified CPE was studied by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The effect of the scan rate on the cyclic voltammetric response of 50.00 \( \mu \text{mol l}^{-1} \) riboflavin at the modified electrode surface is shown in Fig. 2. The relation between the peak current, \( \Delta I_p \), and the square root of scan rate, \( \sqrt{v} \), was linear for both cathodic and anodic waves in the range of 25.00–400.00 mVs\textsuperscript{-1}, indicating that the reduction and reverse oxidation of riboflavin at the modified electrode MnTPP/CPE was a diffusion controlled process.\textsuperscript{33}

Voltammetric studies showed that riboflavin undergoes two electron exchange reactions. The remarkable current enhancement in the electrochemical reaction of riboflavin occurs when the electrode is modified with MnTPP. On the other hand, there is no measurable wave for the modified electrode in the potential range studied in plain supporting electrolyte. This behavior, which has been observed at several potential scan rates and different pH of supporting electrolyte, clearly demonstrates that the MnTPP acts as electrocatalyst in electrochemical response of riboflavin on the surface of modified electrode. The calibration graph constructed under the optimal conditions was linear in the concentration range of 0.01 to 10.00 \( \mu \text{mol l}^{-1} \) and the detection limit (3\( \sigma \)) of 8.00 \( \text{nmol l}^{-1} \) was obtained for determination of riboflavin. Figure 3 shows differential pulse voltammograms of different concentration of riboflavin on the surface of modified electrode MnTPP/CPE.\textsuperscript{34}

The authors have also studied the effect of the modifier percentage, so different differential pulse voltammetry measurements of a
The RSD for 1.00 μmol 1⁻¹ riboflavin was successfully applied for the electrocatalytic determination of riboflavin in different matrices, such as cow milk, non-alcoholic beer, B-complex and multivitamin tablets. The recovery levels from the analyzed samples were in the range of 98.00%–101.40%, indicating that the constituents of the samples do not significantly interfere with the determination of riboflavin.

Zarei, Jamali and Bagheri modified the CPE by incorporating an ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate (HMHP), to the carbon paste for the determination of riboflavin in pharmaceutical formulations. The ionic liquid was used as a binder in the carbon matrix to form the homogenous paste and to replace the classical paraffin oil or silicon oil. The composition of the HMHP consists of highly conductive ions and represents a major advantage in the design of CPE. CPEs modified with the ionic liquids show many advantages over the classical devices, like, the increasing rates of increased sensitivity, reduced volatility, high conductivity, and electron transfer. During the analysis, a pre-concentration step was introduced, for riboflavin to be absorbed on the sensor’s surface. The adsorbing process makes the proposed sensor to become disposable. With the optimal parameters selected the calibration curve was built. The linear response was observed in the 0.04–1.50 μmol 1⁻¹ concentration range, which was followed by a decrease in sensitivity at higher concentrations. A low limit of detection of 11.00 nmol 1⁻¹ was calculated for a signal-to-noise ratio of 3.00. The stability of the electrode was evaluated by probing of the electrode response to electrocatalytic oxidation of 0.10 μmol 1⁻¹ of riboflavin after 15 days and the current response preserved nearly 94.30% of its initial values. The interference study includes different species like vitamins B₁, B₆ and B₁₂, folic acid, diclofenac sodium, ascorbic acid, diphenhydramine, and ibuprofen tested in a mixed solution which contained riboflavin under optimal experimental conditions, and any of the proposed species did not interfere in the determination of riboflavin. The proposed method was suitable for the determination of the concentration of riboflavin in pharmaceutical formulations with a mean recovery percent 96.11% (n = 3).

Tigari and collaborators investigated the optimum sodium lauryl sulfate concentration used in the preparation of a modified carbon nanotube paste electrode (SLSM/CNTPE) applied in the electroanalysis of riboflavin. The addition of the surfactant to the bare electrode matrix exhibited a high sensitivity (1.04 A L mol⁻¹) and a low detection limit (92.50 nmol 1⁻¹). The SLS modifier was found to be extremely sensitive to acidic and basic media and it also had reduced the over potential for the reduction/oxidation of the analyte in contrast with the unmodified electrode. The good obtained stability was studied by cycling 30 consecutive runs and observing that 90.10% of the initial current signal was retained even after 30 cycles, a good reproducibility for 5 individual measurements was obtained with a RSD value of 3.03% and the repeatability for 5 successive measurements was obtained with a RSD value of 2.01%. Pharmaceutical samples were analyzed for the application of the method and the average recovery obtained was 98.00% with a RSD value of 1.53%. The authors have concluded that the designed sensor showed a good alternative for riboflavin determination besides other previously reported dates.

The comparison of several parameters of riboflavin for the all above mentioned CPEs is summarized in Table I. The lowest limit of detection was 8.00 nmol 1⁻¹ when in the carbon paste matrix was incorporated manganese (III) tetraphenyl porphyrine (MnTPP) as a modifier. Regarding the materials discussed as modifiers, it can be suggested that in the future studies of the determination of riboflavin, the MnTPP should be used in the construction of the new electrochemical sensors.

**Glassy carbon electrode (GCE) used for determination of riboflavin.**—Glassy based electrodes allow measurements in a...
wide potential range, even at more negative values compared to platinum or gold electrode. Glassy carbon electrode possess a fast electrokinetics due to their high electron transfer rate on the electrode surface and the response is faster compared to thin-film metals electrodes, which can be associate with the large diameter of the working area. Based to its amenable surface, the GCE can be easily modified by electropolymerizing inert organic monomers or by coating protective polymers on the top of the electrode with Nafton. To form a stable bond between the Nafton and the GCE surface, the Nafton can be applied to the surface directly or electrochemically. The main advantage of the GCE is the possibility to be reused by polishing the surface.

Owing to its physical and chemical properties, glassy carbon has become an interesting and widely applied electrode and possess a rather high chemical inertness. Until 2014 it was no report about a glassy carbon electrode (GCE) coated with a nanostructured conducting polymer used in the determination of riboflavin. In that period of time a graphene film deposited on a GCE for the electro-oxidation of riboflavin was developed.

Nie and coworkers have proposed poly(3,4-ethylenedioxythiphene)-PEDOT films and these films were prepared by incorporation of two electroactive species, ferrocene carboxylic acid (Fc−) and fericeric acid (Fe(CN)$_6^{3−}$) as doping anions during the electropolymerization of PEDOT at GCEs from aqueous solution. Such nanostructured films combined the advantages of PEDOT (high conductivity and stability) together with electroactive species (good electrochemical activity) and they were applied as electrochemical sensors for simultaneous determination of vitamins. The electrochemical response of riboflavin increases linearly with the increase of the target biomolecule concentration. For the three PEDOT proposed sensors PEDOT/ClO$_4$−/GCE, PEDOT/Fc-/GCE and PEDOT/Fe(CN)$_6^{4−}$/GCE the linear ranges were 0.15−300 μmol l$^{-1}$, 0.1−300 μmol l$^{-1}$, and 0.04−200 μmol l$^{-1}$, respectively; whilst the limit of detection was 80.00 nmol l$^{-1}$, 50.00 nmol l$^{-1}$, and 20.00 nmol l$^{-1}$, respectively. As it can be seen, the lowest limit of detection was obtained using PEDOT/Fe(CN)$_6^{4−}$/GCE (20.00 nmol l$^{-1}$) and the steady-state current of riboflavin (10.00 μmol l$^{-1}$) was reached within 8 s. The proposed electrodes displayed good superiority in terms of linear ranges and limits of detection. No change in the current response was observed when testing the PEDOT/ClO$_4$−, PEDOT/Fc− and PEDOT/Fe(CN)$_6^{4−}$ modified GCEs for the detection of riboflavin, in the presence of various common ions, such as Cu$^{2+}$, Fe$^{3+}$, Zn$^{2+}$, Na$^{+}$, Cl$^{−}$, K$^{+}$, Mg$^{2+}$, SO$_4^{2−}$, Ca$^{2+}$, CO$_3^{2−}$, NH$_4^{+}$, NO$_3^{−}$ and some physiological interferences, like uric acid, urea, glucose, oxalate, glycine, alanine, L-cysteine, L-tyrosine, L-glutamine, L-serine and valine by DPV. Stability was an another advantage of the PEDOT/ClO$_4$/GCE, PEDOT/Fc−/GCE and PEDOT/Fe(CN)$_6^{4−}$/GCE, which was tested by measuring the response current decay during repetitive CV cycling and storage at room temperature. After 20 successive determinations of riboflavin, the current response signals at PEDOT/ClO$_4$/GCE, PEDOT/Fc−/GCE, PEDOT/Fe(CN)$_6^{4−}$/GCE retained almost 100.00% of its original activity. The storage stability of the sensors was also investigated in the same solution after storage for 7 days, the oxidation potential for riboflavin was unchanged and the current response signal decreased about 1.52% riboflavin of their initial response at PEDOT/ClO$_4$/GCE, 1.85% at PEDOT/Fc−/GCE and 1.74% at PEDOT/Fe(CN)$_6^{4−}$/GCE. The simple fabrication procedures, wide linear ranges and low detection limits for repeated detection measurements suggested that these modified GC electrodes can be good and attractive candidates for practical applications.

Hajian et al. reported in 2014 the template electrolysint and characterization of polyythionephene nanotubes, and sensing application of them towards the oxidation of riboflavin, since the performance of sensors and biosensors can be improved by using nanomaterials. Conduction polymer nanotubes have been synthesized chemically or electrochemically in the pores of a template. Electrochemical deposition in the pores of the template has attracted a good deal of research interest because with this method it is easy to control the length, diameter, shape and structural properties of the nanotubes. In this study, the authors used conductive polymer nanotubes for GCE modification (PTN/GCE) which exhibited a very good activity towards riboflavin detection and determination. The modified GCE had a sensitivity of 1.03 μA/μmol l$^{-1}$, in the concentration range of 0.01−65.00 μmol l$^{-1}$, and possessed a detection limit of 3.00 nmol l$^{-1}$ (signal-to-noise ratio of 3). The efficient electrocatalytic property of PTN/GCE towards riboflavin could be attributed to the large catalytically electroactive surface area of nanotubes. The percentage value of RSD for 10.00 μmol l$^{-1}$ riboflavin by using ten fresh modified electrodes obtained was 4.30%, which shows high repeatability of the electrode modification method and high stability (after three weeks). The interference studies were carried out with several species like various common metal ions (Na$^{+}$, Mg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$), vitamins (ascorbic acid, vitamin B$_1$, vitamin B$_6$, vitamin B$_12$) and other organic compounds (folic acid, uric acid) and the results showed no serious interference. The practical application of the developed sensor showed an average recovery of 97.00% in human plasma, suggesting a high accuracy of the PTN based sensor. The method provides a useful tool for very sensitive and accurate determination and quantification of riboflavin in human plasma.

Kaur and Srivastava fabricated a modified glassy carbon electrode based on nanocrystalline zirconosilicates/titanosilicates/aluminosilicates for the simultaneous electrochemical oxidation of vitamins. Zeolites have attracted an increasing interest in the construction of sensors and biosensors because of their high active surface area, porous nature and easy functionalization of the surface with organic groups. Nanocrystalline zeolite significantly improved the sensing capability when it was compared to bulk zeolite material. Nanocrystalline zirconosilicate (Nano-Zr-ZSM-5/GCE) exhibited the highest activity towards riboflavin, with a linear range from 0.03−500.00 μmol l$^{-1}$, sensitivity values of 2.80 μA/μM l$^{-1}$ cm$^{-1}$ and lower detection limit of 5.00 nmol l$^{-1}$. The fabrication method was highly reproducible, the RSD value (five Nano-Zr-ZSM-5/GCE electrodes) was found lower than 2.60%. The modified electrode had excellent stability after 20 days and it was highly selective, the possible interferences of some inorganic ions (K$^+$, Na$^+$, Cl$^{−}$, HPO$_4^{2−}$, H$_2$PO$_4^{−}$, Ac$^{−}$, Ca$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Mg$^{2+}$), and organic compounds (ethanol, glucose, ascorbic acid, uric acid, urea, alanine, L-cysteine, L-tyrosine, L-glutamine, L-serine and valine, folic acid, vitamin E) did not show any changes in the peak current response. In the analytical application the proposed sensor was able to determine the analyte in multivitamin tablets with high accuracy and selectivity even in the presence of other vitamins and multiminerals, recovery levels were in the range from 97.00 to 99.70% with RSD 2.08%. The proposed methodology was simple, rapid and can be easily used in quality control laboratories as an alternative analysis method.

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Table I. Comparisons of linear range and detection limits for the riboflavin with CPEs.

| CPE modified with: | Method | Linear range (μmol l$^{-1}$) | Limit of detection (nmol l$^{-1}$) | Sample (reference) |
|-------------------|--------|-----------------------------|-------------------------------------|------------------|
| Co$^{2+}$ − Y zeolite | CV     | 1.70−34.00                  | 710.00                              | multivitamin and B complex tablets.30 |
| MnO$_2$          | DPV    | 0.02−9.00                   | 15.00                               | vitamin B$_2$ tablets.32 |
| MnTTPP           | DPV    | 0.01−10.00                  | 8.00                                | pharmaceutical and food samples33 |
| HMHP              | DPV    | 0.04−1.50                   | 11.00                               | pharmaceutical formulations35 |
| SLS               | CV     | 2.00−200.00                 | 92.50                               | multivitamin capsule37 |
Sumathi et al., investigated the electrochemical behavior of riboflavin by square wave voltammetry (SWV) on a glassy carbon electrode modified with α-Fe3O4/MWCNTs/AuNPs. The authors used Fe3O4 nanodendrites due to their importance in crystallography research and wide applications in nanosensors,40 microdevices41 with high degree of structural anisotropy and highly active facets of their surface.40 The gold nanoparticles (AuNPs) enhanced specific interactions between the receptors and analytes and have made the measurements highly selective and sensitive.40 Using SWV method, the linear range was obtained between 0.3 μmol l−1 to 6.00 × 10−3 μmol l−1, with a sensitivity of 6.00 mmol l−1 and a good resolution coefficient of 0.9956 for α-Fe3O4/MWCNT/AuNPs/GCE. The high sensitivity and the distinguishable separation of the oxidation peak currents provide a good opportunity for the precise determination of riboflavin at α-Fe3O4/MWCNTs/AuNPs/GCE. The anti-interference test by SWV of the α-Fe3O4/MWCNTs/AuNPs electrode for the detection of riboflavin was evaluated and no remarkable change was observed for the foreign species like: L-dopa, serotonin, epinephrine, cysteamine, dopamine, tyrosine and K+, Ca2+, NO3−, NH4+, Cl−. The modified electrode is highly selective towards the determination of riboflavin even in the presence of these interferents with an RSD value of 4.85%. In the term of stability, riboflavin is strongly adsorbed on the modified electrode surface after repeated electrochemical continuous cycling in 100.00 μmol l−1 of riboflavin solution. By measuring the peak current obtained from six different GCEs modified electrodes showed an RSD value of 3.75%, confirming that the results are reproducible. The analytical performance of the α-Fe3O4/MWCNTs/AuNPs modified electrode was applied for riboflavin determination in pharmaceutical and milk powder samples using SWV technique with satisfactory recovery. All advantageous features like sensitivity, stability, selectivity and reproducibility, make the proposed sensor applicable in medical, food or other areas.40

Bagoo and Nandibewo have fabricated, characterized and found the application of the graphene-GCE in electro-analytical determination of riboflavin. Graphene film upon casting onto the surface of GCE, developed a good electrochemical interaction with riboflavin. The π-like structures of the isooxalazine ring in riboflavin and sp2 carbon atoms in graphene were the major sources to shuttle the electron between riboflavin and graphene. The determination of riboflavin was carried out by using DPV as it produced current-voltage curves with better intensity and sharp peaks at lower concentration of riboflavin. Under optimum conditions, the good linear relationship between peak current and concentration of riboflavin was in the region from 1.00 mmol l−1 to 1.50 × 10−3 mol l−1 and the peak current increased with the increase in concentration of riboflavin. The LOD and LOQ were calculated, 1.00 × 10−3 mol l−1, respectively 3.43 × 10−6 mol l−1. The repeatability and reproducibility of the modified sensor was examined by repeating five experiments on the same day and in the same standard conditions and over 2 days from different standard solution of riboflavin. The RSD values of peak potentials (0.91%) and peak currents (1.13%) between day reproducibility are almost identical to that of within a day if the temperature was kept almost constant which could be ascribed to the excellent stability and reproducibility of graphite-GCE. The effect of some common excipients (citric acid, lactose, dextrose, starch, gum acacia, glucose, sucrose and tartaric acid) used in pharmaceutical formulation was examined and these did not interfere with the voltammetric signal of riboflavin. The precision of the method was evaluated for the analysis of pharmaceutical assay of riboflavin sample using 5 different aliquots of stock solution. The present method showed good recoveries (98.70%), low RSD (2.82%) and bias values such as 1.30%. The detection results of five urine samples were obtained with good recovery range from 96.00 to 103.00% and RSD value was equal to 1.49%. The proposed method could be applicable in the pharmacokinetic studies as well as clinical and quality control laboratories.41

Madhuvilakku and his group developed a novel one-pot environmentally friendly facile nano architecture biosurfactant synthesis strategy for flower like Fe3O4 anchored reduced graphene oxide (Fe3O4/rGO) sheets concurrently with improved physico-chemical properties for supercapacitors and sensor applications. In the current developments, reduced graphene oxide (rGO) issued as the substitute for pristine graphene because of their high surface area and high electroconductivity due to the restoration of conductive carbon conjugated networks,52 which has been favorable for the accurate detection of the biological analytes. In addition, rGO also contains different oxygen vacancies, which make it feasible to be modified with others nanomaterials or biological molecules to further improvements in its chemical and physical properties for the next generation point-of-care biosensors and energy storage devices.53 The Fe3O4 nanostructures over the rGO sheets were used due to the excellent electrical, thermal, mechanical properties, enlarged specific surface area and distinctive electrical conductivity. The two linear calibration curves ranged from 0.30 μmol l−1 to 1.00 mmol l−1 and 1.00 μmol l−1 to 100.00 μmol l−1 with correlation coefficient of 0.9930 and 0.9850, respectively. Dual linear range in the determination of riboflavin is due to the adsorption of the reduction products of riboflavin at the electrode surface.54 For the first linear range the detection limit of the modified electrode towards riboflavin determination was calculated to be 89.00 nmol l−1. These results indicated that the Fe3O4/rGO/GCE electrode is more sensitive for the detection of riboflavin than other reports in the literature. The common ions such as Na+, Ca2+ and some physiological interferents such as glucose, dopamine, uric acid, ascorbic acid and glycine did not significantly interfere with the riboflavin detection, manifesting high selectivity of the proposed biosensor. After running 25 continuous cycles, a negligible loss of approximately 2.50% in the current signal was noticed and retaining 97.50% of the initial current response thereby indicating efficient stability and anti fouling properties of the Fe3O4/rGO/GCE electrode for riboflavin reduction. The reproducibility of the riboflavin sensor was investigated by detecting 1 mmol l−1 riboflavin at three freshly prepared modified electrodes. All three electrodes exhibited similar current responses and an RSD value of 2.30% was obtained indicating that the electrochemical behavior of the Fe3O4/rGO/GCE is highly reproducible. Long-term storage stability of the modified electrode was also studied and the results proved that it has excellent storage stability. The long-term operational stability of the fabricated sensors is attributed to the structural stability and robustness of the prepared Fe3O4/rGO composites. Based on the DPV response of pharmaceutical samples, the recovered values were in the ranges between 97.20% and 104.00%. The estimated amount of riboflavin by this proposed modified electrode was in excellent agreement with riboflavin amount present in pharmaceutical products along with the acceptable RSD value. Also, the obtained results indicated that the Fe3O4/rGO/GCE modified electrode could useful for the detection of riboflavin presence in pharmaceutical samples.55

Sonkar and his group have prepared a nanocomposite of Mn(salen)Cl (where salen is N,N-bis(salicylidene)ethylenediamine) and multiwalled carbon nanotubes (MWCNTs). The advantageous properties of MWCNTs and catalytic properties of Mn(salen)Cl (represented as MnIII,salen) are combined in this new nanocomposite (represented as MWCNTs-MnIII,salen). The main objective of this work was the simultaneous determination of riboflavin and vitamin B6 in the presence of each other. DPV technique was applied for the simultaneous determination of riboflavin and vitamin B6 on GCE/ MWCNTs-MnIII,salen electrode in 0.1 M pH 7.0 phosphate buffer solution. A good sensitivity coefficient equal to 82.90 μAmM−1 cm−2 was observed for GCE/MWCNTs-MnIII,salen electrode and the limit of detection for riboflavin was found to be 730.00 nmol l−1. To evaluate the selectivity of the GCE/MWCNTs-MnIII,salen, some possible interferences were tested in the determination of riboflavin (0.1 mmol l−1) by an amperometric method. The results showed that 0.10 mmol l−1 of vitamin B3, MgCl2, uric acid, glucose, NaCl, alanine, or gluconic acid gave negligible interferences for the determination of riboflavin. The equal amount (0.1 mmol l−1) of folic acid (vitamin B9) and riboflavin (0.1 mmol l−1) provide considerable
interference, found to be 7.1%, which conclude that the presence of vitamin B₆ can affect the determination of riboflavin. The decreasing of interference effect can be improved by standard addition calibration method. These results demonstrated a high selectivity for riboflavin analysis, using the proposed sensor. The RSD value (3.70%) for riboflavin determination on GCE/MWCNTs-MnIII-salen indicates the excellent reproducibility and durability of the modified electrode. The oxidation signal measured by DPV technique for riboflavin (0.10 mm) at GCE/MWCNTs-MnIII-salen decreased by 3.00 and 90.90% respectively. The GCE/MWCNTs-MnIII-salen has efficient potential for the electrocatalytic determination of riboflavin with high reproducibility and stability. The recovery results of this method on GCE/MWCNTs-MnIII-salen were satisfactory for riboflavin determination as follow, the ranges of recoveries percentage obtained for Optineuron injection was calculated to be 94.70%–98.60%, and for Beplex Forte tablet was 90.90%–97.50%, respectively. According to the good results obtained, MWCNTs-MnIII-salen can be used as a potential electrocatalyst or sensor for simultaneous determination of riboflavin and vitamin B₆.

Selvarajana, Suganthia and Rajarajan have fabricated an electrochemical sensor based on a novel ZnO-manganese hexacyanoferrate nanocomposite (ZnO/MnHCNF)—modified GCE for detecting riboflavin in 0.1 M PBS (pH 6.40) for the first time using DPV. Manganese hexacyanoferrate (MnHCNF) have attracted attention of scientists because both the manganese and iron elements have two oxidation states, (II) and (III), respectively. MnHCNFs have wide applications in molecular magnets, optomagnets, humidity sensors, electrochromic devices as well as electrocatalysis. MHCNFs have been widely used to modify electrode as an electron transfer mediator in electrochemical sensors and biosensors. ZnO is one of the most important functional materials because it has versatile applications that are attributed to its excellent properties such as wide direct band gap, large binding energy, excellent chemical and thermal stability, and good piezoelectric properties.55 In the field of bio-sensing, ZnO has gained much attention because of its unique properties such as high catalytic efficiency, strong adsorption ability, bio-compatibility and high isoelectric point. The DPV current linearly increased when the concentration of riboflavin was increased from 0.20 μmol l⁻¹ to 3.00 μmol l⁻¹ in the ZnO/MnHCNF-modified GCE with a correlation coefficient of 0.9970. The detection limit was found to be 0.01 nmol l⁻¹. Various biological compounds (thiamine, nicotinamide, vitamin B₆, vitamin B₁₂, vitamin C, folic acid, ascorbic acid, uric acid, glucose and lysine) and several metal ions (Fe³⁺, Mg²⁺, Ca²⁺, K⁺, and Na⁺) did not significantly interfere with riboflavin detection. The repeatability of the ZnO/MnHCNF—modified GCE examined with an RSD of 3.67%, revealing good response. The sensor retained 94.23% of its original response after 10 days, which decreased to 89.23% after 60 days, indicating its good stability. All of these results revealed that the ZnO/MnHCNF—modified GCE has acceptable reproducibility and long-term stability (up to 8 weeks), which make it attractive for the preparation of biosensors. The practical application of the ZnO/MnHCNF-modified electrode was tested by measuring the concentration of riboflavin in pharmaceutical and milk powder samples with good results. All advantages like sensitivity, stability, selectivity, reproducibility and freedom from interference make the proposed sensor applicable in medical, food or other fields.56

In 2018, Sriramprabha et al. have described the preparation of SnO₂/rGO nanocomposite by a simple hydrothermal process and used for the selective detection of riboflavin. Tin oxide (SnO₂) is well known material, due to the involvement of suitable oxidation states and its higher catalytic activity in sensor applications. The schematic representation of material preparation and their electrochemical evaluation is given in Fig. 4. This nanocomposite was used to modify GCE and employed for electrochemical detection of riboflavin through CV, ASV and SWV techniques. For the SnO₂/rGO/GCE nanocomposite the SWV responses exhibited a significant linearity in the range of 0.1–150 μmol l⁻¹. This is due to the domination of interfacial electron transfer reaction facilitated by the active redox sites on the rGO matrix (Scheme 2). The developed SnO₂/rGO/GCE sensor has been highly sensitive and selective towards riboflavin determination in the presence of various common interferences like L-Tryptophan, L-Proline, L-Cysteine, L-Lysine, L-Glutamic acid, L-Ascorbic acid, Glucose, Cholesterol, Dopamine Hydrochloride, Uric acid, Thiamine, Sodium sulfate and Ammonium chloride. The stability of the electrode was analyzed for 30 days. The current responses corresponding to different analyte concentrations (0.1–150 μmol l⁻¹) were obtained on SnO₂/rGO/GCE stored in phosphate buffer for 30 days and compared with the results obtained on day 1. From the outcomes, the current response was retained almost 99% (the maximum S.D = 0.0277) for distinct analyte concentrations even after 30 days. All result demonstrated the accuracy of developed electrode in riboflavin sensing with notable linearity, selectivity and sensitivity with a LOD of 34 nmol l⁻¹. The improved electrocatalytic efficacy of the SnO₂/rGO/GCE was recognized through remarkable recovery above 98.5% of riboflavin in the pharmaceutical compounds and energy drinks that can be conceded in real-time applications.59

Figure 4. Schematic representation of material preparation and its electrochemical performances in riboflavin detection.59
The comparison of several parameters of riboflavin for some GCEs is summarized in Table II. The lowest limit of detection was 0.01 nmol l\(^{-1}\) when GCE was modified with ZnO-manganese hexacyanoferrate nanocomposite (ZnO/MnHCNF). Regarding the materials discussed as modifiers, in future projects, the ZnO-manganese hexacyanoferrate nanocomposite should be applied more into the construction of the new electrodes used to detect riboflavin.

**Conclusions**

This review summarizes some of the most recent electrochemical determination of riboflavin from pharmaceutical products, food or human plasma, because it is a worldwide need to be able to use a fast method to determine the riboflavin levels from a variety of samples. The electrochemical sensors used were based on carbon paste or glassy carbon modified with different electrocatalytic materials. The choice of electrochemical sensor for the determination of riboflavin should also be influenced by the type of real samples that are used in the applications. For the practical applications high selective and reliable methods of analysis are required, that can be able to cover wide concentration ranges and able to reach low concentration limits for riboflavin. The advantages such as high sensitivity, stability, selectivity and reproducibility make the electrochemical sensors discussed in this review to be seen as potential tools for analysis of riboflavin in medical, pharmaceutical, food or other fields.

The carbon-based electrodes presented in this review demonstrate a fast electrochemical detection of riboflavin on the modify electrode surface with a fast kinetic process and also a great electrocatalytically activity. Furthermore, the material design and synthesis used in the detection of riboflavin are focused on the minimization of the fouling effect of the electrode surface which may lead to a biological application.

**Table II.** Comparisons of linear range and detection limits for the riboflavin with GCEs.

| GCE modified with: | Method | Linear range \(\mu\)mol l\(^{-1}\) | Limit of detection nmol l\(^{-1}\) | Sample (reference) |
|-------------------|--------|-------------------------------|-----------------------------------|-------------------|
| PEDOT/ClO\(_4\)\(^{-}\) | DPV    | 0.15–300.00                   | 80.00                             | orange juice\(^{18}\) |
| PEDOT/Fe\(^{2+}\) | DPV    | 0.1–300.00                    | 50.00                             |                   |
| PEDOT/Fe(CN)\(_6\)\(^{4-}\) | DPV    | 0.04–200.00                   | 20.00                             |                   |
| Conductive polymer nanotubes | DPV | 0.01–65.00                   | 3.00                              | human plasma\(^{43}\) |
| Zeolite            | DPV    | 0.03–500.00                   | 5.00                              | multivitamin tablets\(^{45}\) |
| \(\alpha\)-Fe\(_2\)O\(_3\)/MWCNT/AuNPs | SWV | 0.30–6.00 \(\times\) 10\(^{-3}\) | 6.00                              | pharmaceutical products\(^{50}\) |
| Graphene film      | DPV    | \(1.00 \times 10^{-3}–15.00 \times 10^{-3}\) | 0.10                              | urine\(^{41}\) |
| Fe\(_2\)O\(_3\)/rGO | DPV + CV | 0.30–1.00                   | 89.00                             | pharmaceutical and nutrition products\(^{55}\) |
|                   |        | 1.00–100.00                   |                                   |                   |
| MWCNTs-Mn\(^{III}\)salen | DPV | 1.00–400.00                   | 730.00                            | pharmaceutical\(^{46}\) |
| ZnO/MnHCNF         | DPV    | 0.20–3.00                     | 0.01                              | pharmaceutical and nutrition products\(^{36}\) |
| SnO\(_2\)/rGO      | SWV    | 0.10–150.00                   | 34                                | multivitamin pharmaceutical compounds and energy drinks\(^{59}\) |

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