THE COMPLICATIONS AND DIAGNOSIS OF HYPERBILIRUBINEMIA: A REVIEW

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Abstract

Bilirubin is majorly a product of RBC turnover. The heme group of hemoglobin is catalyzed by the action of microsomal heme oxygenase to produce biliverdin which is further acted upon by biliverdin reductase to form bilirubin. A majority (~99.99%) of the unconjugated bilirubin binds to albumin for transportation in blood. Bilirubin levels in serum are an indicator of liver health an increase in the serum bilirubin beyond normal values i.e., < 0.3 mg/dl is noted is called hyperbilirubinemia. Hyperbilirubinemia may lead to severe neurological damage in newborns. Hyperbilirubinemia may be diagnosed by comparing the levels of free and total bilirubin in the blood. The present review details the complications of hyperbilirubinemia and various methods of its diagnosis.

Keywords: Bilirubin, Hyperbilirubinemia, Hemoglobin, Biosensors, Diagnosis, Serum.

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1. Brief Background

1.1 Bilirubin

Bilirubin is commonly known as bile pigment that is secreted as bile juice from the liver and is involved in raising the pH of the food bolus before it enters into the small intestine. The pigment imparts a yellow color to the stools. The compound is majorly a product of RBC turnover i.e., produced as a result of hemoglobin degradation and nearly 20% is synthesized from other heme-containing proteins such as myoglobin. Total turnover of the bilirubin per day is 4 mg/kg body weight.[1]

The heme group, removed from the hemoglobin or other heme-containing protein molecules, enters the porphyrin catabolism pathway and is processed to form yellow- dark orange-colored bilirubin in two steps. In the first step, microsomal heme oxygenase catalyzes the breaking of the heme ring to produce green-colored biliverdin, and in the second step, the biliverdin is reduced to bilirubin by the action of nicotinamide adenine dinucleotide phosphate (NADPH) dependent enzyme, biliverdin reductase. The bilirubin thus, formed is then conjugated with glucuronic acid before excretion.

2. Chemistry of bilirubin and related Physiology

The IUPAC name of bilirubin is 3-[2-[[3-(2-carboxyethyl)-5-[(Z)-(3-ethenyl-4- methyl-5-oxopyrrol-2-ylidene)methyl]-4-methyl-1H-pyrrol-2-yl]methyl]-5-[(Z)-(4- ethenyl-3-methyl-5-oxopyrrol-2-ylidene)methyl]-4-methyl-1H-pyrrol-3-yl]propanoic acid. The molecular formula of the compound is $C_{33}H_{36}N_4O_6$ with molecular mass 584.7and the structural formula is shown in figure 1. The compound belongs to class biladienes, with linear tetrapyrrole consisting of dipyrrole rings. It has two carboxylic acids as the functional group. The physio-chemical properties of the molecule can be attributed to its internal six hydrogen bonds.[2]

2.1. Physiology of bilirubin

Bilirubin is secreted from the liver as bile juice. The quantity of bile is an indicator of liver health.[3] The bilirubin is present in blood or other body fluids either as free bilirubin or complexed with albumin or conjugated with glucuronic acid etc. [4, 5]The normal values of total bilirubin in the blood are up to 1.2 milligrams per deciliter in adults and 1 mg/dl in children while conjugated (direct) bilirubin should be < 0.3 mg/dl. The condition in which an increase in the serum bilirubin beyond these normal values is noted is called hyperbilirubinemia.

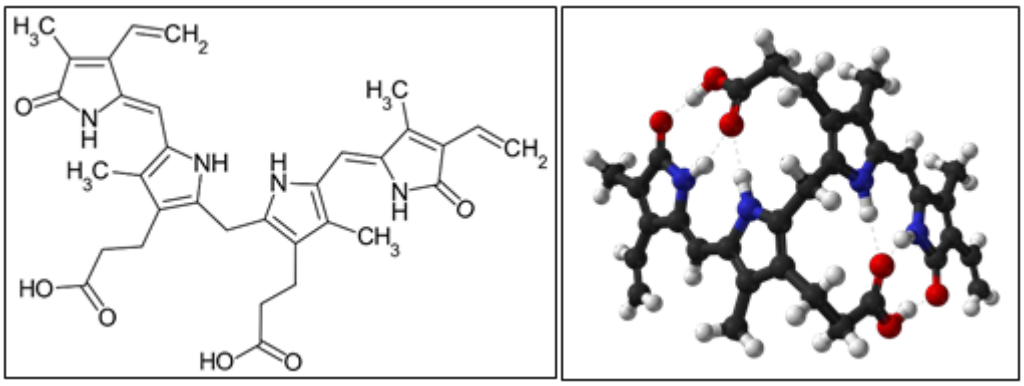


Figure 1. Structure of bilirubin

A majority (~99.99%) of the unconjugated bilirubin binds to albumin for transportation in blood. Transportation of free bilirubin in the blood is in traces. The solubility of the free bilirubin is pH-dependent and it may deposit in the tissues.[6] The free bilirubin is very important since it determines the bio-availability of the conjugated bilirubin to the tissues.[2]

The conjugated bilirubin when reaches the liver, pigment disassociates from the album to enter the hepatocytes.[1] The transport of bilirubin from the liver sinusoid to the hepatocytes may involve a protein receptor-mediated endocytosis or by simple diffusion. The former case is concentration gradient independent while the latter is concentration gradient dependent, i.e., the flow of bilirubin can be reversed in case the concentration is more inside. The bilirubin pigment that manages the entry to the hepatocytes is extracted into the periportal space. The rest of the pigments are reassociated with the albumin and enter the sinusoidal flow and are finally excreted via urine.[1]

3. Epidemiology of hyperbilirubinemia

The conjugated bilirubin acts as an antioxidant and prevents damage due to oxidative stress. Though, in the case of hyperbilirubinemia, severe neurological damage has been reported in neonates.[2] Newborn babies, esp., preterm newborns are highly susceptible to hyperbilirubinemia. Nearly 80% of the preterm newborns have hyperbilirubinemia, while 60% of the full-term newborns have this condition .[7] In some cases of hyperbilirubinemia in neonates, the bilirubin may cross the blood-brain barrier and may cause irreversible damage to brain cells. The condition is termed acute bilirubin encephalopathy. Acute bilirubin encephalopathy may result in insult and injury of CNS, auditory, visual, dental, neuromotor, and language impairments.[8]

The acute bilirubin encephalopathy condition can be diagnosed by comparing the levels of free and total bilirubin in the blood. The condition is characterized by high levels of free bilirubin but normal total bilirubin levels in jaundiced newborns.[7] Total bilirubin levels were not distinct, when tested in infants with a low birth weight, with or without bilirubin-mediated deafness, in a phototherapy study conducted by the National Institute of Child Health and Human Development.[9] Because of the overlap of the total bilirubin in the control and test, it is not a reliable candidate to diagnose the condition. The use of total bilirubin for detecting acute bilirubin encephalopathy gives a number of false positives. [10–13]

Severe neonatal hyperbilirubinemia and kernicterus, although used interchangeably in practice, are different from each other. As per Karimzadeh et al. [14], "Acute manifestations of bilirubin neurotoxicity in early stages in the neonatal periods is, defined as Acute Bilirubin Encephalopathy (ABE) and permanent and chronic sequela of bilirubin toxicity is known as kernicterus." Incidence of kernicterus in patients need not necessarily had chronic bilirubin encephalopathy condition in past. Similarly, chronic bilirubin encephalopathy may or may not develop into kernicterus.

Hyperbilirubinemia may also prove fatal, especially in the countries with low resource settings, where neonatal jaundice is not diagnosed or treated on time. Neonatal jaundice is highly common.[15] During pregnancy, the bilirubin of the fetus is removed by the mother. However, post-parturition, the neonate has to excrete the bilirubin itself and most of the time the system is not well-developed. The post-natal imbalance between the bilirubin production and excretion is not a cause of worry, except in the case of production of bilirubin isomer 4Z,15Z unconjugated bilirubin IXα. [7] The isomer acts as a neurotoxin and when its concentration increased beyond a certain point may cause acute bilirubin encephalopathy or seldom kernicterus.[10] Acute bilirubin encephalopathy occurs in nearly 1 % of the preterm newborns and 15% of the full-term newborns with hemolytic disorders. [16, 17]

Rh incompatibility between the Rh-negative mother and Rh-positive newborn is an important contributor to jaundice cases.[18] The Rh and ABO incompatibility between mother and neonate increases the severity of hyperbilirubinemia. The severity of hyperbilirubinemia leading to bilirubin-induced encephalopathy is a cause of concern. Despite the advancement of modern care, the cases of Rh incompatibility led bilirubin-induced encephalopathy are on the rise.[14] Though, treatment with exchange transfusion was found effective for lowering the incidence in the case of the ABO and Rh incompatibility .[16]

The factors responsible for unconjugated bilirubin to cross the blood-brain barrier include conditions like asphyxia, acidosis, etc. Physiological polycythemia, short-lived RBCs, less absorption of bilirubin by hepatocytes, deficiency of receptor proteins or UDP-glucuronosyl transferase enzymes in the preterm neonates. At times, over-active b-glucuronidase, deficient glucose -6-phosphate dehydrogenase, reduced gut flora, ante-or-post-natal infections in the neonates, or breast milk jaundice are other factors associated with hyperbilirubinemia in neonates. Hyperbilirubinemia can disturb the buffering capacity of the blood resulting in the formation of a neurotoxin, bilirubin acid.

4. Need for diagnosis

The literature was searched on Pubmed® at https://pubmed.ncbi.nlm.nih.gov/. The search with the keyword 'Hyperbilirubinemia' yielded 32,584 results (Figure 2a). The curve obtained is a bimodal curve with sudden increase in the year 1964 and 1965 with 864 research papers in 1965. By 1980, the papers per year for the term was again normalized. The number of publications again increased from the year 2002 and reached 818 in the year 2020 and still the peak is not reached yet. Similar trend was observed when the PubMed was searched using the keywords, '(hyperbilirubinemia) and (diagnosis)'. The

PubMed gave 16487 results with these keywords (Figure 2b).

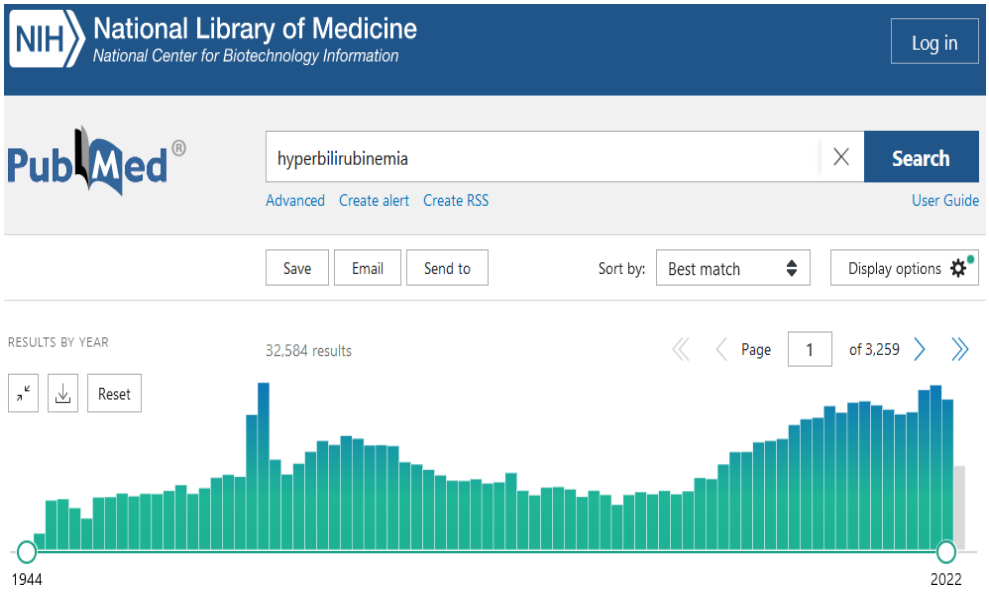
Detection of jaundice early in the life of neonates is highly important for the prevention of bilirubin-induced encephalopathy. [19, 20] Bilirubin-induced encephalopathy is more common in developing countries than in developed countries because of their absence or lack of facilities. Poor families in the developing world rely on the visual detection of jaundice. Visual detection involves observing the yellowness of the whites of eyes or skin which is not so reliable method of detection.[21]

5. Available methods for bilirubin detection

5.1. Total cutaneous Bilirubin

Total cutaneous bilirubin measurement relies upon phototherapy i.e., observing the bilirubin under the skin by exposing the skin to the blue light in the range of 420-to- 470-nanometer wavelength. The total cutaneous bilirubin estimation is accurate, reliable, robust, non-invasive, quick, and cost-effective. The accuracy of the method is compromised in the case of dark-skinned neonates and the test cannot be used for decision making.

A.



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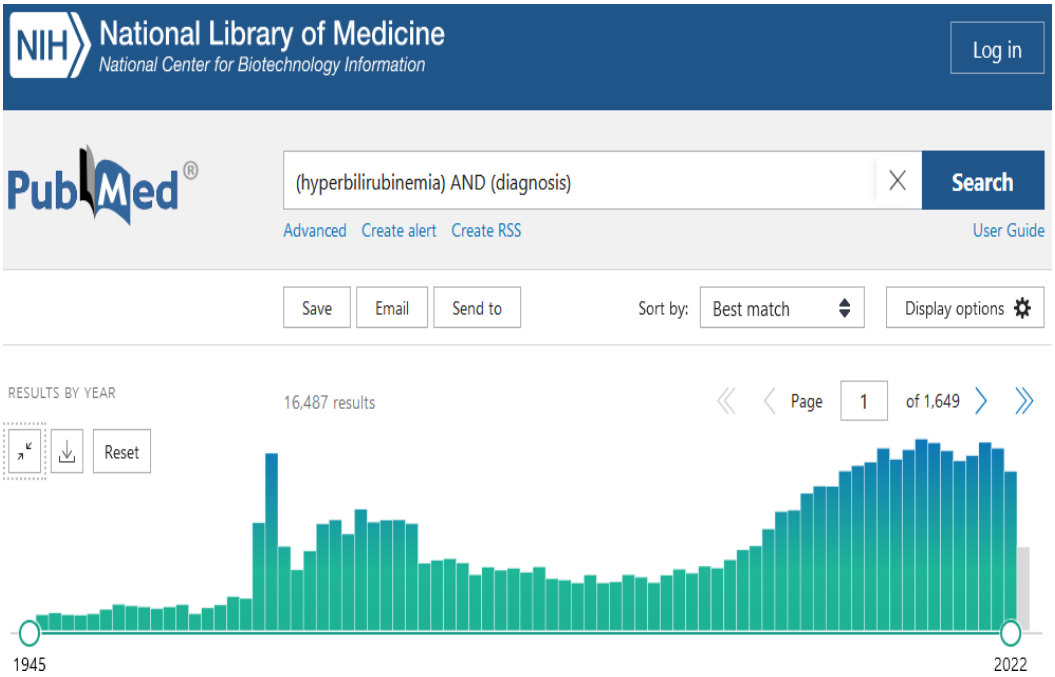

Figure 2. Number of research papers appeared in PubMed® search with the keyword (a) '(hyperbilirubinemia)', and (b) '(hyperbilirubinemia) and (diagnosis)'

5.2. Total Gas Bilirubin

The method requires very less sample ranging from 30 to 100 L. Blood gas bilirubin measurement can be carried out using the Co-oximetry principle. The method is fast since does not require any centrifugation of the samples. A one-two-one correlation of bilirubin measurements using co-oximetry and serum bilirubin with the diazo method was done by Lano et al.[22] . Their results revealed that the bilirubin estimation using co-oximetry on the Radiometer was comparable with those obtained by the diazo method. The co-oximetry may be seen as the next alternative to the gold- standard serum bilirubin assays.

5.3. Total Serum Bilirubin

Total serum bilirubin is generally quantified using Malloy and Evelyn [23] method or Jendrassik and Grof[24] method. Both these methods are based upon Van den Berg's reaction principle in which, bilirubin reacts with diazotized sulphanilic acid to form a pink to purple colored azobilirubin. The product can be read at 540 nm. The conjugated bilirubin being soluble readily gives the colored product (i.e., within 30 seconds) while the conjugated bilirubin takes time (5 minutes to 30 minutes depending upon the solubilizers, such as methanol, used). The tests are not accurate for the measurement either of conjugated or unconjugated bilirubin.

Another traditional popular method for bilirubin estimation was the peroxidase method.[25] The method relies upon horseradish peroxidase mediated (by hydrogen peroxide or ethyl hydrogen peroxide) oxidation of free bilirubin. Since the horseradish peroxidase cannot bind with conjugated bilirubin, the test is used to determine the concentration of unbound or free bilirubin. [25, 26] The estimation requires absorbance at 440 nm and 460 nm. At 440 nm the rate constant is determined by observing a decrease in the optical density of unbound bilirubin in the absence of binding proteins, and then, at 460 nm the unbound bilirubin is determined using the value of the rate constant determined earlier by noting down a decreased in the optical density of the unbound bilirubin the presence of the binding proteins. The test gives total bilirubin as well as free bilirubin concentration in plasma[27] as well as a tissue culture medium .[28]

6. Biosensors

Biosensors are meant for estimating the rate of a reaction especially, biological reaction depending on certain electrical or fluorometric signals that quantum of which corresponds to the analyte concentration.[29] A biosensor must exhibit selectivity, reproducibility, stability, sensitivity, and linearity to qualify for being a worthy biosensor.

The concentration of an acidic analyte can be correlated with the electric signal in terms of electric potential was first demonstrated by M. Cremer[30] , three years before the concept of hydrogen ion concentration as pH expounded by Sørensen[31] . It took another 12 years for the invention of an electrode for the first pH meter by Hughes [32] in 1922. L.C. Clark [33], the father of biosensors, developed the first biosensor for the detection of oxygen in 1953, and then for the detection of glucose in 1962.[34] Following this, an electrode for the detection of urea was also developed.[35]

The biosensors may be categorized into different types of biosensors based upon the bioreceptor or the transducer they use. The different categories are enzyme-based biosensors, microbe or cell-based biosensors, tissue-based sensors, immune sensors, DNA sensors, magnetic biosensors, thermal biosensors, colorimetric biosensors, piezoelectric biosensors, fluorescent-based biosensors, optical biosensors, etc.

7. Biosensor based diagnostics

Biosensor-based diagnostics of hyper bilirubinemia include fluorimetry[36], voltammetry, and amperometry (monitoring redox reaction of BR at an electrode's surface).[37, 38] The biosensors used for bilirubin have been reviewed by Hooda et al.[39] .

7.1. Flourimetry

The fluorimetry can be used to develop fluorescent biosensors by mounting one or more probes on small scaffolds of receptors to the analytes. The bilirubin oxidase combines with a fluorescent derivative to yield the conjugated enzyme, which can be read at 487 nm and 520 nm upon excitation. The fluorescent signals correspond to the concentrations of the analyte.[40, 41] Gene cloning and expression methods enabled the synthesis of recombinant proteins coupled with fluorescent markers such as green fluorescent protein, GFP. This enabled the workers to express the fluorescence within cells. Single chain Förster resonance energy transfer (FRET) biosensors have two auto- fluorescent proteins, which when coming in proximity, due to interaction with two proteins, exchange fluorescence resonance energy between themselves.[40, 42]

Brown et al.[43] used hematofluorometer to determine the blood concentration of free and conjugated bilirubin in a cohort of 79 newborn infants. The group also reported the use of automated front-face fluorometry in 28 newborns in the same year .[44] The results obtained by using front-face fluorometry were reported to be comparable to the results of diazo, Sephadex gel filtration, and peroxidase-oxidation methods.

Fluorometry-based diagnosis of hyperbilirubinemia was first reported by Santhosh et al.[36] . They used human serum albumin stabilized gold nanoclusters of nearly 1nm as a probe. Bilirubin interacts with the human serum albumin resulting in the quenching of the fluorescent nanoclusters. The authors reported the sensitivity of the method to 248 ± 12 nM. The authors recommended a temperature range of 25-50°C and a pH ranging from 6.0 to 9.0 units. Xiao et al.[45] used Langmuir-Blodgett which was human serum albumin-gold nanoclusters functionalized by glucuronic acid forestimation of unconjugated bilirubin in human serum samples. The use of glucuronic acid increased the binding sites for bilirubin on the nanocluster. The fluorescent sensor could detect up to 1nM of unconjugated bilirubin with anti-interference activity.

Ellairaja et al.[46] developed a florescent platform using a newly-synthesized fluorescent compound, bromopyridine-2,3-diyl) bis(azanylylidene)) bis-(methanylylidene diphenol). The platform was sensitive enough to detect as low as 1 pM and up to 500 µM with a limit of detection of 2.8 pM at pH 7.4. The imine compound-based sensor was very sensitive and accurate. The sensor could detect the bilirubin in urine as well as the serum samples. Moreover, the sensor could be used for the colorimetric samples as well.

A fluorescent biosensor for bilirubin detection was fabricated using europium- doped yttrium oxide nanosheets .[47] The yttrium oxide modification of the europium- doped nanosheets had higher luminescence than that of the europium alone .[48] The europium-doped nanosheets were also modified with 2-thenoyltrifluoroacetonate or 2-acetylbenzothiophenetrifluoroacetone and coated with polystyrene.[47] 2-thenoyltrifluoroacetonate modified europium-doped yttrium oxide nanosheets were the most sensitive and reliable biosensor for the detection of unconjugated bilirubin as compared with the other two biosensors.

7.2. Amperometric and Potentiometric Biosensors

7.2.1. Based upon enzyme, bilirubin oxidase

An amperometric biosensor relies upon the charge produced as a result of the redox reaction. The rate of the flow of charge is proportional to the concentration of the analyte. Thus, the total electric current is read as a measure of the analyte concentration. An amperometric biosensor may or not use an enzyme as a catalyst for the redox reaction. Two redox enzymes, namely bilirubin oxidase, and horseradish peroxidase can be used for the enzymatic amperometric biosensors.

An amperometric biosensor was designed using two oxidases, namely, bilirubin oxidase and horseradish peroxidise .[49] These two enzymes were incorporated in the graphite-epoxy matrix. The resulting biosensor was renewable upon polishing. The limit of detection of the biosensor was 4 µM and the linear detection was up to 100 µM.

Immobilization of the enzymes increases their efficiencies due to reusability .[50] Multilayer enzyme electrodes were also constructed for immobilizing bilirubin oxidase enzyme covalently .[51] For this purpose, 3-mercaptopropionate ester layer was coated on a gold surface. The biosensor had the stability of >3months when stored at 4°C. The albumin, however, was also found to be a confounding factor for the electric current produced.

An amperometric biosensor based upon bilirubin oxidase immobilized on an oxygen electrode for bilirubin detection was also developed .[52] The electrode measured a decline in the oxygen concentration during the action of bilirubin oxidase enzyme on bilirubin. The electrode produced a linear curve up to 2060 mM. The electrode was better than its predecessors since no interference was observed due to albumin in the serum.

Glassy carbon electrodes were modified using multiwalled carbon nanotubes and the enzyme bilirubin oxidase was immobilized on the surface by covalent bonding .[53] The modification of the glassy carbon electrodes was found to add to the reduction potential of the electrode. Further, the biosensor recoded the data using linear sweep voltammetry, which is a singly linear sweep from lower to upper potential limits. Similarly, in another attempt, an electrode of silica sol-gel and carbon nanotube were used to immobilize bilirubin oxidase to construct an amperometric

biosensor for bilirubin detection .[54] Bilirubin oxidase enzyme was immobilized on different layers of aromatic compounds enveloping multi-walled carbon nanotubes modified gold electrodes either by adsorption or by covalent linkage[55] to understand the effect of the aromatic compounds on the efficacy of the electrodes. The authors reportedpyrroloquinoline quinone was found to be the most suited aromatic compound.

A hybrid biological fuel cell has a bacterium, *Shewanella oneidensis* as anode terminal and the enzyme bilirubin oxidase as cathode terminal .[56] The bilirubin oxidaseenzyme was immobilized on a carbon nanotube electrode using silica sol-gel coating.

Bilirubin oxidase enzyme was immobilized on zirconia coated silica nanoparticles (SiO2@ZrONPs)/chitosan (CHIT) composite electrodeposited onto Au electrode .[57] The biosensor showed optimal activity at pH 8.5 and temperature 35°C and the estimation was very quick i.e., within 2 seconds. The biosensor worked for 120 days with a LOD limit of 0.1 nM and a linear range of 0.02–250 μM. On similar lines, the group developed another biosensor based upon the immobilization of the bilirubin oxidase enzyme on graphene oxide nanoparticles decorated polypyrrole layer electrochemically deposited onto the fluorine-doped tin oxide glass plate to develop an amperometry biosensor for bilirubin detection .[58] The biosensor had Ag/AgCl as standard electrode and platinum as the auxiliary electrode. The amperometric sensor had a LOD limit of 0.1nm and the detection range is 10nM to 500μM. The amperometric sensor with immobilized bilirubin oxidase on graphene oxide nanoparticles has a half shelf life of 150 days .[58]

Nanoparticles of organic molecules ensure a greater surface area of the molecule. The formation of nanoparticles of enzymes results in increased enzymatic activity, increased stability, and enhanced biocompatibility. The enzyme nanoparticles can be immobilized on both organic and inorganic supports with the help of covalent linkage to the support. The immobilized enzyme nanoparticles are more productive and reusable with high precision and accuracy .[59] The nanoparticles of bilirubin oxidase were immobilized on a polyethylene film to design an amperometric biosensor for bilirubin estimation .[60] The immobilized enzyme nanoparticles had a Km value of 0.015 μM and a Vmax value of 2.56 μmol/mL/min. The biosensor showed linearity of results between 20nM to 250μM. The bilirubin estimation was found correlated with the estimation using colorimetric assays.

7.2.2. Biosensors based upon enzymes other than bilirubin oxidase

Nanoparticles of zinc sulfate were prepared and catalase enzyme was immobilized on them to fabricate a biosensor for bilirubin detection .[61] The biosensor gave a linear relationship at the anode in the range of 3 μM to 50 μM with a limit of detection of 2 μM. The temperature and pH requirement for the biosensor was 25°C andpH 7.0 respectively.

7.2.3. Inorganic catalysts

The specific detection of biological organics without the use of an enzyme is challenging. A portable amperometric potentiostat was fabricated by forming a thin layer of bilirubin imprinted poly-(methacrylic acid-*co*-ethylene glycol dimethacrylate) onto the gold layer .[62] The biosensor was moderately accurate but fairly high precision. Rahman et al. [63] fabricated an amperometric bilirubin biosensor by coating ascorbate oxidase containing polyethyleneimine film over a conductive poly-terthiophene-Mn(II) complex. The bilirubin detection by the biosensor was based upon the Mn(II) ion- mediated electron transfer. The biosensor yielded a linear calibration plot ranging from 100nM to 50μM and a lower detection limit of 3.8nM. The biosensor was stable andhad a response time of less than 5 sec.

Manganese-Copper complex was electrochemically deposited on the Nafion electrode to fabricate a non-enzymatic amperometric biosensor .[64] The Mn-Cu/ Nafion electrode catalyzed two-electron oxidation of dipyrromethane moiety to dipyrromethene in bilirubin. The range was 1.2 μM to 420 μM and the detection limit was ~25nM.

Nanoparticles of Fe3O4-hydroxyapatite-Polypyrrole were fabricated on the magnetic glassy carbon electrode.[65] The biosensor was rapid and worked in the range 100nm to 17μM with a detection limit of 7nM. The biosensor can detect bilirubin in the serum samples.

Reduced graphene oxide-polystyrene was coated on to glassy carbon electrode to develop a non-enzymatic amperometric biosensor for bilirubin.[37] The biosensor hada linear range of up to 450 μM with a detection limit 2 μM.

Porous transparent titanium dioxide was fabricated on a glass substrate by the sol-gel

method.[66] The modified molecularly imprinted polypyrrole was imprinted on the porous transparent titanium dioxide. The limit of detection for the biosensor was 2mM and a linear range of 30 nM to 28 µM and can be detected as low as 1 nM.

Nanocomposites of two elements, Copper oxide - cadmium oxide were fabricated on glassy carbon electrodes for amperometric detection of the serum bilirubin.[67] The biosensor showed a linear correlation in the range of 10 pM to 10 mM with a high correlation score of 0.9347. The limit of detection was as low as 1 pM.

Innovations in screen printing enabled the controlled printing of carbon electrodes. Multiwalled carbon nanotubes or graphene were deposited on the screen- printed carbon electrodes to fabricate two electrochemical biosensors for the detectionof bilirubin in human serum .[68] The biosensor measured the electrocatalytic activity during the conversion of bilirubin to biliverdin at +0.25V and biliverdin to purpurin at +0.48V. Between the two screen-printed biosensors, the multiwalled carbon nanotube- based biosensor had a higher detection limit (~0.3nM) than the graphene-based biosensor (~0.1nM), while the graphene-based biosensor had higher sensitivity and a broader linear range of 100 nM to 600 µM against 500 nM to 500 µM in the case of multiwalled carbon nanotube-based biosensor.

Ceria nanocubes were fabricated on screen-printed electrodes to synthesize a biosensor for unconjugated bilirubin detection in serum samples .[69] The ceria nanocubes exhibited high catalytic abilities and were highly stable. The detection linear range was 1 to 100 µM with a limit of detection of 100 nM in buffer and 1 µM in serum samples. Volume as low as 5 µL of human serum is sufficient to estimate the unconjugated bilirubin.

7.3. Potentiometry

A nano graphite-based Platinum microelectrode to voltametrically measure unconjugated bilirubin .[70] In the addition to unconjugated bilirubin, the biosensor can also detect the presence of uric acid and ascorbic acid in the serum due to different oxidation peaks in voltammetry. Moreover, the readings of bilirubin were not affected by the interference of uric acid and ascorbic acid in the serum. The bilirubin detection was also independent of the presence of albumin.

Carbon electrodes were screen printed using graphite carbon ink for the voltammetric estimation of unconjugated bilirubin .[71] The screen-printed carbon electrodes can be used to detect ascorbic acid, uric acid, dopamine, glucose, creatinine, and ethanol. The biosensor can be used to estimate the affinity of the unconjugated bilirubin to albumin.

8. Conclusions:

Hyperbilirubinemia is serious in neonates if goes undetected. Several methods are known to diagnose hyperbilirubinemia that include non-invasive cutaneous detection, total serum bilirubin, and total gas bilirubin. Traditional gold-standard diagnosis of hyperbilirubinemia relies upon the biochemical estimation of the serum bilirubin concentration. Advances in the techniques to manipulate the biomoleculessuch as enzyme immobilization, screen printing, and nano-formulations of enzymes, have led to the formulation of several biosensors.

Conflict of Interest

Authors have no conflict of interest.

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