The amperometric cholesterol sensors biology essay

Science, Biology

T Kappers, BSc PJ Porte, BScAbstractThis review paper gives an overview of amperometric sensors for cholesterol developed duringthe last five years. Cholesterol is a chemical found in fat, blood and cells and can causeheart attacks when levels are too high. Most sensors are based on the same reactions which isCholesterol + O2ChOx ????????????????! Cholest ???? 4 ???? en ???? 3 ???? one + H2O2. Several sensors are viewed andthe important working methods and features are described. This paper shows there are severaldifferent methods for the detection of cholesterol, all with their own features. IntroductionCholesterol is a chemical substance found in fat, blood, and cells in the human body and higheranimals. It is essential for human's life sinceit can build and maintain membranes of cell. Unfortunately, high cholesterol levels are relatedto cardiovascular disease such as myocardial infarctionand stroke. 2 Its level in blood is animportant parameter in the diagnosis and preventionof disease. Ideally, the total cholesterolconcentration in a healthy person's blood shouldbe less than 200 mg/dL (<5. 17 mM). The borderlinehigh is defined as 200-239 mg/dL (5. 17-6. 18 mM), and the high value is defined as above240 mg/dL (> 6. 21mM). 8 Most cholesterol sensorsare based on the enzymatic reaction in theuse of cholesteroloxidase (ChOx) which can bedescribed as in equation 1 and can be seen infigure 1. Cholesterol + O2ChOx ????????????????! Cholest ???? 4 ???? en ???? 3 ???? one + H2O2(1)Because of the increasing amount of people withhigh levels of cholesterol, the cholesterol sensorbecame more important. The consequence is thedevelopment of new cholesterol sensors. Mostlythe developed cholesterol sensors are amperometricbiosensors. The amperometric biosensorsare chosen because of their good selectivity, rapid

response and low cost. 8 This paperreview describes the developed amperomet-Figure 1: Reaction of cholesterolric cholesterol biosensors of the last five years, mostly based on the reaction described above. AmperometryAmperometry is a technique that uses electriccurrent to determine a concentration of a specificion. A reduction current, which occurs bythe flow of electrons from the electrode to thesolution, causes a reduction reaction. The reductionreaction is part of the redox reactionwhich occurs in the solution. This reaction willcause a decrease of the electrode potential. Theelectrons needed for this reaction are from a reactionat the reference electrode. Because of thisreaction less ’oxidation’ ions are present aroundthe working electrode. This causes a gradientin the ion concentration, which causes a diffusionof the specific ions. There will arise a flux1to the electrode, which is related to the reducingcurrent. The original concentration of theions can be determined through the reductioncurrent. The reduction current is dependent onthe flux and the flux is dependent on the concentrationgradient as can be seen in equation 2. ired = nFADox_Cox(x)_x(2)With a few assumptions this equation can besimplified. First of all, the slope of the gradientis linear. Secondly, the layer where the diffusiontakes place has a fixed thickness. Thirdly theCox(x = 0) has to be zero, for that a good potentialhas to be chosen. The result of equation 2and the assumptions above is shown in equation3. ired; limiting= nFADox_(Cox(bulk) ???? Cox(x = 0))_x

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nFAD_xCox(bulk)(3)With equation 3 it is possible to determine theconcentration of ions. SensorsFor this review paper there is looked at

varioussensors for the detection of cholesterol. Thesesensors are described briefly and the importantfeatures are summarized in table 1. RSD isthe sensor to sensor reproducibility relativestandard deviation. Abdelwahab et al. used the conductingpolymer, poly3, 4-diamine-2, 2, 5, 2-thertiphene(PDATT) as a matrix for the immobilizationof biomolecules and Gluthatione (GSH) as amatrix for the capture of gold nanoparticles(AuNPs) and biomolecules (ChOx). 1 In thisstudy the process of immobilized ChOx on theAuNPs-GSH/PDATT electrode was studied todevelop a cholesterol sensor. The maximumcurrent response was observed at a potentialof -0. 3 Volt with an optimum pH of 7. 0 andan optimum temperature of 25°C. The performanceof the cholesterol sensor was tested underoptimum conditions and showed a sensitivity of0. 054 _A_M???? 1, as can be seen in figure 2, witha linear range from 10-30 _M and a detectionlimit of 0. 3 _M The detection time was 0. 5s= _M with a RSD of 4. 7%. Figure 2: Amperometric response usingthe ChOx/MP/AuNPs-GSH/PDATT electrode. The inset shows thecorresponding calibrationplot of cholesterol. 1Most cholesterol sensors use cholesterol oxidase(COD) as the enzyme to catalytically oxidizecholesterol to 4-cholesten-3-one and hydrogenperoxide. Disadvantages of this use arephysical and chemical influences like temperatureand pH effects. Chiang et al. detect cholesterolbased on indirect electrochemical oxidationwith bromine which acts as electron mediatorsor electrocatalysts. 2 A three-electrodesystem was used, with a platinum plate as workingelectrode, a Ag/AgCl/saturated KCl electrodeas reference electrode and a platinum plateas counter electrode. It takes 60 seconds toreach an equilibrium and an equilibrium potentialof 0. 5 V was reached.

The cholesterolis oxidized in a electrolytic solution consistingof NaClO4, KBr and N-N dimethyl-formamide(DMF) in which NaClO4 acts as a supportingelectrolyte to improve the conductance. KBrserves as a source of the bromine and DMF isfor dissolving cholesterol. Amperometric detectionwas chosen at an applied potential of 1. 82V (vs. Ag/AgCl/saturated KCl). With thischolesterol sensor there was found a sensitivityof 31. 08 _Ammol???? 1cm???? 2, a linear range of 30-200 _M, a detection limit of 3. 2 _M and a responsetime of 45 seconds. The sensitivity ofthis cholesterol sensor showed in figure 3. Figure 3: Calibration curve for the amperometricdetection of cholesterol using an indirect electrochemicaloxidation method. 2The cholesterol sensor of Fang et al. has acarbon electrode base for the working, referenceand auxiliary electrodes. 3 More ingredientsshould be added to the working electrode, like cholesterol esterase, cholesterol dehydrogenaseand the coenzyme nicotinamide adeninedinucleotide (NAD). The reactions that takeplace can be seen in figure 4. This sensor isdisposable. A buffer of 50 mM phosphate with a pH of7. 0 is used to get a required pH for a sensorresponse that is as high as possible. The sensoris tested for different temperatures. It showedthat there is only a small different in sensorresponse between a temperature range from 15to 42°C which shows this sensor is temperatureindependent. The sensor has a linearity range of 50 - 500mg/dl and a detection limit of 50 mg/dl. Thesensitivity is equal to 0. 02344 _A=(mg= dl). This is showed in figure 5. The sensor hasa stability of 100 days. The sensor tests thecholesterol through a simple test strip. It hasa few advantages because of its simplicity likethe low costs and the reproducibility of thefabrication of the

strips. Because it is disposableit can easily be used in every household. Figure 4: Schematic depiction of total cholesterolbiosensor, where CHD, cholesterol dehydrogenase; CE, cholesterol esterase; Chol, cholesterol; ChE, cholesterol ester; PDo is theoxidation state of PD and PDr is the reductionstate. 3The amperometric cholesterol sensor ofHuang et al. is a non-enzyme sensor based onthe host-guest effect of Beta-cyclodextrin(_-CD) for cholesterol. 4 This host-guest effect isused to extract the cholesterol out of a specificsolution. An Au electrode is used as base for theworking electrode. The total working electrodewas named as MB/_-CD/AuNPs/Au. Methyleneblue(MB) and Beta-cyclodextrin are addedto the electrode. The sensor uses a saturatedcalomel electrode as reference electrode andplatinum wire as auxiliary electrode. As shown in figure 6 the cholesterol replacedthe MB which was in the _-CD. And becauseof this change the peak current decreases. Thismeans that the MB molecules diffuse into thesolution, which results in a reduction of theredox reaction. The disadvantages of an enzymatic sensor, likethat it is required to retain the catalytic activityof the enzyme, will be overcome by the use of a3Figure 5: the calibration curves of the totalcholesterol biosensors at sampling time 38 s withlinear regressions. Five readings were taken ateach measurement. 3nonenzymatic sensor. The highest sensor response was reached with apH of 7. That is why 20 mM phosphate bufferedsaline (PBS) with a pH of 7. 0 was chosen as thesupporting electrolyte. When this sensor is placed in a cholesterol solutionthe MB will be replaced by the cholesterol. Because the MB is in the solution there will bea reduction of the redox signal. The sensor showed a linearity from 2: 0 _ 10???? 8to 5: 0 _ 10???? 5 M and has a minimum

detectionlimit of 7 _ 10???? 9 M. The recovery of the determinationof the sensor has been measured toknow if the sensor could be used for real sampledetermination. The recovery has a range of96. 5 to 103. 6% for three serum samples, thisshows that this technique can be used for thedetection of cholesterol. Figure 6: Schematic presentation of the fabricatedcholesterol amperometric biosensor. 4The amperometric cholesterol sensor of Liet al. uses porous tubular silver nanoparticlesto measure cholesterol. 5 This will be used forthe working electrode with a glassy carbonelectrode. The reference electrode is a saturatedcalomel electrode and the counter electrode isplatinum wire. It is a nonenzymatic sensor. An enzymatic sensor has a few disadvantageslike that the activity of an enzyme decreasesbecause of the use and the sensor response isdependent of the temperature and pH. Thatis why also a nonenzymatic sensor is made toovercome these disadvantages of an enzymaticsensor. And the porous Pt nanostructure shouldbe working well for this electrode. The response can change through changesin pH, the potential and the amount of Agnanoparticles on the working electrode. At apH of 13 the current was at his maximum. Theoptimal potential is +0. 35V. And the amountof nanoparticles should be 1. 5_L. The sensor has a linear range from 2: 8_10???? 4M to3: 3_10???? 2M and a detection limit of 1: 8_10???? 4M. This linearity is shown in figure 7. The sensorhas a good stability, after 50 measurements thesensor response was still 95. 3%. Its responsetime was 20 seconds. Figure 7: Amperometric responses of 1D poroustubular Ag modified GCE at 0. 35V upon successiveadditions of 5. 0_L 0. 28M cholesterol to5. 0mL 0. 1MpH 13 NaOH. Inset: plot of catalyticcurrent vs cholesterol concentration (RSD: 4. 51%). 5Ohnuki et al. developed an

amperometricsensor based on hybrid organic-inorganicLangmuir-Blodgett films. 6 The integration oforganic and inorganic materials is promising fordevelopment of new sensors and may produce4new functions. The Langmuir-Blodgett methodused for the development of this sensor providesin ultra-thin multi-layer films in which thicknesscan be controlled for several purposes in sensors. In new sensors for detection glucose theLangmuir-Blodgett is already used, Ohnuki etal. shows that with the same technique a biosensorfor the detection of cholesterol can be made. They developed a hybrid Langmuir-Blodgettfilm of positively charged octadecyltrimethyhlammonium(ODTA), which will immobilize thenegatively charged cholesterol oxidase (ChOx), and nano-sized Prussian blue (PB) clusters. This results in a hybrid Langmuir-Blodgettfilm of ODTA/PB/ChOx, which works as acholesterol sensor. In the first step, the ChOxcatalyzes the cholesterol oxidation reaction. In the second step, PB clusters catalyze thereductive reaction of H2O2 at a small workingpotential with a redox cycle between PB and itsreduced form of Prussian white. This inducesan electrical current flow proportional to cholesterolconcentration which can be measured withan amperometric measurement. The cholesterolbiosensor was tested by measurement of electriccurrent flow, operating at 0. 0 V (vs Ag/AgCl ina buffer solution of pH 7. 0). The reaction timewas found to be 20 seconds with a stable linearrelationship within the range 0. 2-1. 2 mmol/L. The sensor sensitivity turned out to be 1. 6_Ammol???? 1cm???? 2 for a Langmuir-Blodgett filmof six layers as can be seen in figure 8. The amperometric cholesterol sensor of Pundiret al. is based on an epoxy resin membrane. 7The total working electrode is made of an epoxyresin membrane with

immobilized cholesteroloxidase that was applied on a Pt electrode. Thereference electrode is an Ag/AgCl electrode andthe auxiliary electrode uses an Ag wire. Thereis chosen for an epoxy resin membrane becauseit should have a high affinity for enzyme, hightemperature stability, chemical resistance andlow cost. The potential which should be used is +0. 5V, because of the highest sensor current for thispotential. The optimal pH for this sensor is 7. 0and the optimal temperature is 45°C. The cholesterol is measured out of the serum ofFigure 8: Response current density versuscholesterol concentration for ODTA/PB/ChOxLB films (six layers). For the vertical axis, changes in response current density observed atthe each cholesterol injection are plotted. 6the blood. The cholesterol sensor has a linearityof 1-8 mM. The detection limit lies also at1. 0 mM. And the sensor has a sensitivity of0. 63_A= mM and a response time of 25 seconds. The linearity is shown in figure 9. The stabilitydrops for 50% over 6 months. The coefficientsof variation were 1. 59% and 1. 9% and theselow values say that the method is accurate, reproducible and reliable. The major problem for amperometric detectionis the overestimation of the responsecurrent due to inferences such as carbonicacid. This problem can be overcome withseveral techniques as combination of enzymes ordevising techniques to reduce the interference. Safavi et al. use the properties of gold-platinum(AuPt) alloy nanoparticles for fabrication of acholesterol sensor. 8 AuPt has excellent catalysisand resistance to deactivation due to highsynergistic action between gold and platinum. They developed a sensor by electrodeposition ofAuPt on a glassy carbon electrode with chitosanand ionic liquids. Chitosan is used for the excellentfilm forming capability, biocompatibility, nontoxity,

good waterpermeability and high mechanicalstrength. Ionic liquids are used because5Figure 9: Effect of cholesterol concentration oncholesterol biosensor response based on epoxyresin membrane bound cholesterol oxidase (inset): Lineweaver-Burk plot of 1/I vs 1/ cholesterolof epoxy resin membrane cholesterol oxidase. 7of their wide electrochemical window, high ionicconductivity and good thermal stability. ChOxwas immobilized on the surface of the electrodeby cross-linking ChOx and chitosan which resultedin a ChOx/AuPt-Ch-IL/GCE biosensor. The amperometric response was tested at -0. 1V and a pH of 7. 0, which proved to be the bestafter multiple tests. The biosensor exhibitedtwo linear ranges for 0. 05-6. 2 mM and 6. 2-11. 2mM, with a sensitivity of 90. 7 _Ammol???? 1cm???? 2and a detection limit of 10 _M which can beseen in figure 10. The response time of thesensor was less than 7 seconds with a RSD of4. 2%. long-term stability was also tested with90% of its original response after 30 days ofstorage. The amperometric cholesterol sensor ofWisitsoraat et al. uses a functionalized carbonnanotube(CNT) electrode as working electrode. 9 The reference electrode is made of Agand the auxiliary electrode of Pt. The electrodesare placed in a flow injection microfluidic chipbased on polydimethylsiloxane/glass. The sen-Figure 10: Calibration curve of ChOx/AuPt-Ch-IL/GCE with different concentrations ofcholesterol. 8sor uses a microfluidic system. Its advantagesare a low sample consumption, high samplethroughput and high total analysis capability. The CNT electrode is used because earlierstudies showed that is has a high sensitivity, low detection potential and fast response. The injection volume, the pH of the buffer, the enzyme concentration and the ambienttemperature can influence

the sensor response. The optimal pH range will be between 7. 0-7. 5. The best temperature range is between 20-30°C. In both of these ranges the sensor responseis almost horizontal. The maximum enzymeconcentration is set on 50 U/ml , becauseup to this point the increase is linear. Theamperometric cholesterol detection is used withthe ranges determined above. The cholesterol sensor has a linearity between50-400 mg/dl. The sensitivity is equal to 0. 0512nA/(mg/dl). This is shown in figure 11. Thecorresponding detection limit of this sensor is10 mg/dl. It has a throughput of around 60samples per hour. The amperometric cholesterol sensor of Yanget al. is made of platinum nanoparticles. 10 Forthe working electrode this was added to a carbonnanotube thin film. An Ag/AgCl plate in saturatedKCl solution was used as a reference electrodeand a platinum plate was used as counter6Figure 11: Calibration curve as a function ofcholesterol concentration. 9electrode. It is a nonenzymatic sensor. The advantagesof a nonenzymatic sensor are their stability, simple fabrication and low costs and theirreproducibility. Carbon nanotubes have a highelectrical conductivity. The platinum nanoparticleshave the advantage it has a long term cyclicstability and a Pt NP/CNT electrode can havea wide linearity with a low detection limit andhigh sensitivity. The sensor was performed in 50mM phosphate buffered saline (PBS) with a pHof 7. 0. The highest sensor response was reached with a24 bilayer of the CNT film. Also the highest sensitivityis found at a 24 bilayer. The sensor hasa linearity from 0. 005 to 10 mM with a minimaldetection limit of 0. 0028 mM. It has a sensitivityof 8. 7 muA/(mM cm2). This linearity is shownin figure 12. The sensor has a good stability. No obvious changes were seen in a period of 1month.

ConclusionAmperometric cholesterol sensors are made inseveral different ways with all different workingprinciples and features. Which cholesterol sensoris best differs on the purpose of your use andthe requirements someone is looking for. Cholesterolconcentrations in humans are defined ashigh when the cholesterol sensor is above 240mg/dL (> 6. 21mM). For the detection of choles-Figure 12: Calibration plot for current of PtNP/(CNT)24 bilayer electrode as a function ofcholesterol concentration. Applied potential is0. 7 V. 10terol in human there is need of a cholesterolsensor at least linear in the range of interest. Different sensors in this review paper are notlinear within this range, but can be useful forthe determination of cholesterol concentration infood. The concentration of cholesterol in foodmight be lower than in humans and might requirea better sensitivity for instance. The decisionwhich cholesterol sensor one should use isdependent on the several features which can beseen in table 1. A decision could be made onthe importance of the features for the purposeof the cholesterol measurement. For this review paper there is looked at amperometricsensors, which are the most used sensorsfor the detection of cholesterol. Amperiometriccholesterol sensors are the biggest groupof cholesterol sensors developed in the last fiveyear. For further research it might be of interestto review different type of cholesterol sensorsand compare these different types.