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Predicting Response Mechanism of Free Radicals Using Coloured Petri-Nets

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ABSTRACT

Constant production of unstable molecules in the body beyond a limit result in severe damage of cell proteins, DNA, lipids etc. These set of species are free radicals which try to acquire stable configuration by snatching away the electrons from the surrounding molecules thereby resulting in a chain reaction. This leads to oxidative stress which is a starting point for various life threatening diseases. Coloured Petri-Net (CPN) on the other hand is a modelling tool which is suited and used for synchronization, concurrency and message passing as it is a graphical modelling language. This paper presents a modelling set up of reactions caused by free radicals using CPN tool.

Keywords: Free radicals, Lipid peroxidation, Coloured Petri Nets

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INTRODUCTION

When oxygen interacts with certain molecules in human body, there is a constant production of unstable molecules which try to gain stability by stealing electrons from the surrounding molecules, as a result of which it damages the cell proteins, DNA, lipids, carbohydrates etc. These set of highly unstable species are free radicals which have an unpaired electron and they keep on interacting with surrounding molecules to gain a stable configuration of electrons, resulting in a series of chain reaction at a higher concentration, which exhibits a displacement towards the pro-oxidants due to an increment in oxidative metabolism [1] This results in a stressed condition at the cellular level known as oxidative stress, which occur due to many factors such as radiations, medication, chronic inflammation, toxins, infections, illness, arduous physical activity, exposure to alcohol and insecticides, smoking and poor diet. This can further lead to lipid peroxidation and can cause destructive and life threatening diseases such as cancer, neuro-degenerative, reproductive disorders etc. There are several factors which are responsible for the amount of free radicals being produced.

Free Radicals: Sources & Species

The internal sources are mitochondria, reactions involving transition metals, arachidonate pathways, peroxisomes, phagocytes, xanthine oxidase, inflammation and ischaemia; whereas the external sources of free radicals are environmental pollutants, radiation, ultraviolet light, cigarette smoke, certain drugs, pesticides, anaesthetics, industrial solvents and ozone[2]. These free radicals can be broadly categorized into: Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) and Reactive Sulphur Species (RSS)[3]. ROS are derived from molecular oxygen and react instantly with other free radicals to generate more harmful oxidants. Mitochondria being the major site of production, there are certain other enzymes like peroxidases such as myeloperoxidase, oxygenases such as xanthine oxidase and other oxidases which act on the release of these species[4] ROS can be produced both exogenously from sources such environmental agents, ions, metals, chlorinated compounds, radiation and xenobiotics[5] and endogenously from sources such as peroxisomes, microsomes, inflammation caused by cell activation and cytochrome P450 metabolism, neutrophils and eosinophils [6,7].

RNS are produced under the conditions where there is a generation of superoxide anion. The enzymatic activity of nitric oxide synthases (NOS) produces Nitric oxide (•NO). This NOS transfers electrons from NADPH and also oxidizes L-arginine [8]. NO is consumed for the production of $ONOO^-$ and the presence of peroxidises can consume the end product of NO and end products of nitrite, superoxide anion, hydrogen peroxide (H_2O_2) generating NO_2 [9].

RSS are formed by the oxidation of thiols and disulfides. They are redox-active in nature and consist of sulphur at a high oxidation state, eg. Disulfide, sulfenic acid and thiyl radicals. They result in the inhibition of thiol proteins and enzymes due to the rapid oxidation they undergo [10.]

CPN MODEL

In different systems of application, Coloured Petri-Net (CPN) is suited and used for synchronization, concurrency and message passing as it is a graphical modelling language [11.] The CPN tools support this CPN modelling language. CPN Tools is a package that is based on the concept of CPN and supports it [12]. CPN graphical representations are similar to the informal drawings what engineers and designers construct while analyzing and studying any system. Semantics help to implement the simulators for CP nets and forms the initial foundation for analysis. The range of applications where CP-nets can be used vary from informal systems to formal systems. An example of the former can be any description of a work process while an example for the latter can be a protocol used for communication. They may also range from software systems to hardware systems which has several applications. CP-nets build upon a standard concept which is commonly known by system modellers through various programming languages and simple mathematics methods. It is generally short, specific in nature and easy to learn and use [13]. It can provide an explicit description of state and actions, which is opposite and a contrast nature to most of the other description languages which states or act likewise. The reader can change the focus point from one state to other actions [12] Concurrency defines that actions could occur one after the other, be it any order. It is impossible to have two different actions in a same single step is what an interleaving semantics suggests, whereas true concurrency semantics is much easy to work with due to the closer link it has with human imagination about concurrent actions. By connecting and relating many smaller CP-nets together in a well-defined manner, one can construct a larger CP-net. Large model systems can be created, managed and well moduled by hierarchical CP-nets [13]. When debugging of CPN model happens, it can also be analyzed in various ways. It is possible to use automatic simulations. They can be very fast and have great speed with several thousands of transitions per second and at the same time they can represent and be similar to program executions. Time delays can be specified which can represent several action duration in a modelled system, which can make simulations that investigate the system performance [14]. As ones desire and interest, a CPN model can be made, verified and proven. State spaces by which model checking is done can be one of the most direct verification methods for CPN model. An arc can be put up for each transition which is possible from one state to the another and directed graphs with a node for each system of state which is reachable. Sometimes state spaces become large, so it is important to have efficient tools for constructing and for the analysis of state spaces [15]. Work is often done with partial state spaces or state spaces which are in condensed form. Another method of verification is built on place invariants, which is similar to the ones used in verification of the program. This method is more demanding and the required skills of the personnel are limited, thus invariants are used very rarely. But some of the skills can be built into a tool support, and it can become more assessable for practitioners [16]. The states of a CP-net are represented by means of **places** drawn as ellipses. Three places represent the three possible states of free radicals: ROS, RNS and RSS. A state of a CP-net is called a **marking**. It consists of a number of **tokens** positioned on the individual places. Each place has an associated data **type** determining the kind of data which the place may contain.

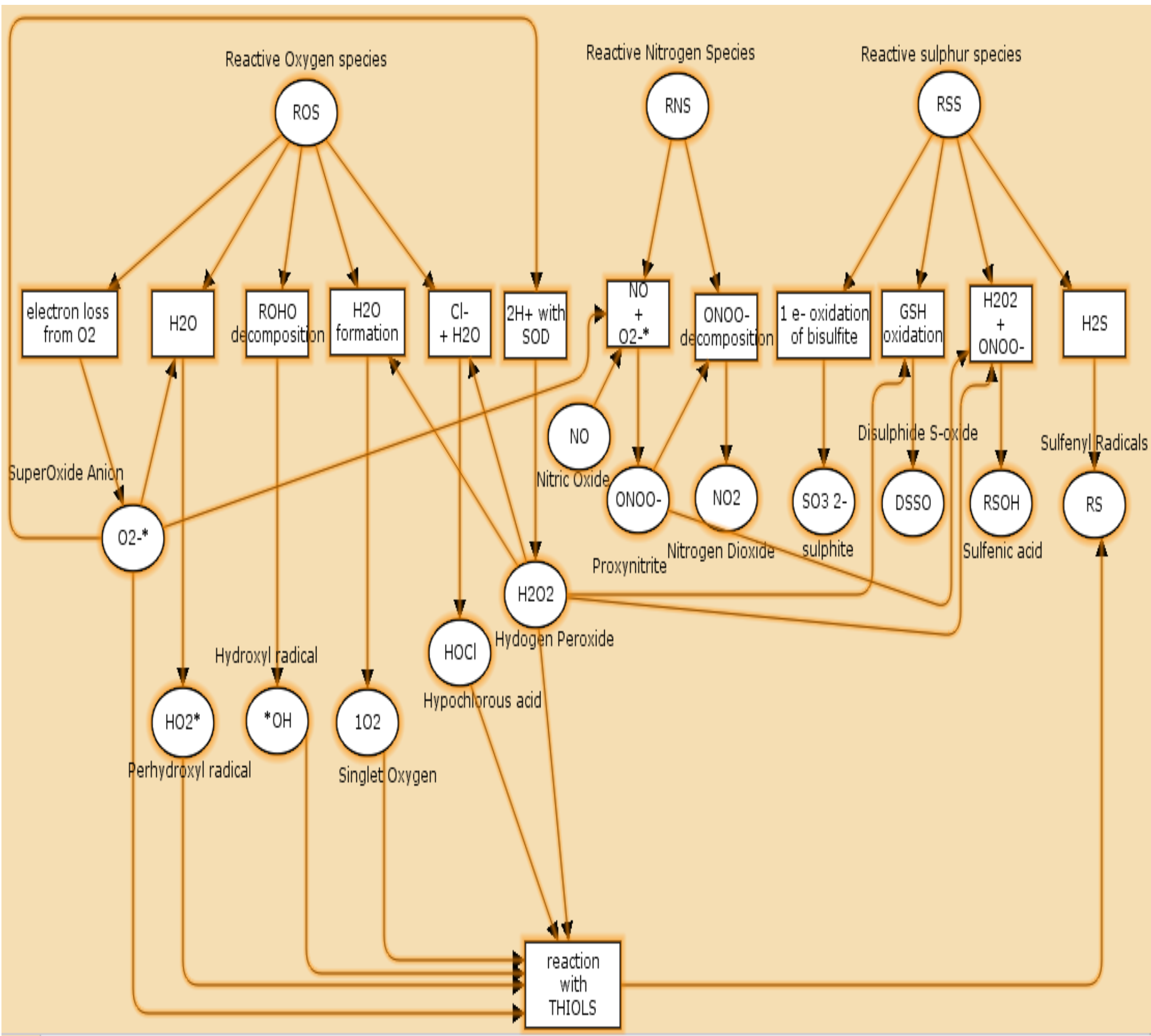

Figure1: Reactive species model using CPN

color ROS = string with O^{2-} |HO_2*| *OH| 1O_2 | HOCl | H_2O_2 ;

color RNS = string with NO | ONOO- | NO_2;

color RSS = string with SO_3^{2-} | DSSO | RSOH | RS;

color RSOH = product H_2O_2 * H_2O_2;

color $ONOO^-$ = product NO * O_2-;

color PROXYNITRATE = list $ONOO^-$;

color SULFENIC ACID = list RSOH;

color REACTION_WITH_THIOLS = record a: ROS * b: RNS * c: RSS;

color FREE_RADICALS = LIST REACTION_WITH_THIOLS;

fun diff(x,y,z) = (x<>y<>z);

var s,r: FREE_RADICALS ;

The initial marking M0 is specified by means of the initialisation expressions which by convention are written with an underline, next to the place. A missing initialisation expression implies that the initial marking of the corresponding place is empty, i.e., contains no tokens. For the places of type ROS, RNS and RSS we have the following initial marking:

M0(Inactive) = ROS, RNS and RSS. M0(Performing) = M0(Waiting) = Ø where Ø denotes the empty multi-set. By convention, we use **FREE_RADICALS** to denote the type, but we also use it to denote the set which contains all data values from the type and the multi-set which contains exactly one appearance of each data value from the type [17].

The free radicals propagated are as:

Free Rad(di) = {(di,d1), (di,d2),, (di,di-1), (di,di+1),, (di,dn-1), (di,dn)}.

Hence we have: M0 (Inactive) = 1`d1+ 1`d2 + 1`dn where the integer coefficients indicate that *Inactive* has one token for each data value di in the type **FREE_RADICALS.**

CONCLUSION

The various reactive species and their generating sources and reasons can be related and it can lead to list out the common factors, using CPN. The model is analysed again after the executable CPN models are developed using the CPN tools package. The passing of the tokens to the places, firing of the transitions and deadlocks can be verified by running the simulation by the user which cannot be illustrated in this paper but rather executing in CPN tool. Events in the CPN model are generated to trigger a sequence of internal events for each individual scenario. Next stage of analysis helps to evaluate concurrency properties of the system in terms of state space diagram and state space reports generated by the CPN tools.

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