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In vivo study of oxidative effect of aspirin nanoparticles in rat blood samples utilizing electrochemical analysis by cyclic voltammetry

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Abstract
The application of nanotechnology in pharmaceutical processing has provided new opportunities for the improvement of the bioavailability and therapeutic efficacy. Pharmacokinetic features of aspirin nanoparticles (ASP-NPs), and their oxidative effects on biological systems are studied in this works. The Oxidative Behavior of Aspirin Nanoparticles in Rat Blood Samples: a Biochemical Look Using Electrochemical Techniques, Cyclic Voltammetry (CV) in Particular the results reveal significant differences between the redox peaks, which imply that oxidizing pressure in the blood was converted to avoiding pressure by the utilization of aspirin nanoparticles. These results are also indicative for safety and redox efficiency of nanoparticle-formulated total drugs, including their implications for blood and gastro-intestinal tract. The investigation was focused on the behavior of nano-aspirin in rat blood, for the determination by cyclic voltammetry at different pHs and temperatures.

Keywords: Aspirin nanoparticles, rat blood samples, oxidation reaction, cyclic voltammetry, *in vivo* study

1. Introduction
Aspirin (acetylsalicylic acid) is a traditional anti-inflammatory drug. and antiplatelet agent. It is a nonsteroidal anti-inflammatory drug (NSAID), used for analgesic, antipyretic, and anti-inflammatory action. [1]. However, the bioavailability of AITC is also poor with significant GI side effects. Nanoparticle drug delivery systems is one of the new drugs delivery strategies to overcome these drawbacks and enhancing the pharmacokinetics and controlled release of drugs such as aspirin are developed [2]. Nanoparticles also pose a threat, as they might be able to induce oxidative stress *in vivo*, mediated by redox cycling, or interaction with cells [3]. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants, which can lead to damage of important biomolecules. [4]. Models that have been developed *in vivo*, such as rodent blood exposure, are also useful to determine the extent of oxidative damage and direct electron transfer reactions ammenable to electrochemical methods, such as CV [5]. CV is a highly sensitive and convenient technique for the determination of redox active constituents in biological matrices. It provides quantitative and qualitative data of the redox reactions, and thus it is widely employed to assess the oxidative stress caused by the drug and nanomaterial treatments [6]. However, its systemic effects, especially as a nanoparticle are not completely understood, and in particular its mechanism for causing oxidative stress have not been well clarified. Delivery of the drugvia nano particulate form may also alter the pharmacokinetics in addition to the bioavailability of aspirin resulting in higher oxidative events in the blood cells [7]. Oxidative stress is a disturbance between reactive oxygen species and antioxidant defenses, and it has been reported to be involved in a variety of pathophysiological disorders such as inflammation [8], cardiovascular diseases [9] and drug induced toxicity [10].
To that end, electrochemical techniques including cyclic voltammetry (CV) have been readily explored because of their ability to offer reliable determination of redox activity in the biological field. Cyclic reactions, making it a valuable method for assessing oxidative effects in blood samples [9]. Voltammetry can be used to directly measure the currents of electrochemical species and has an advantage of monitoring redox reactions sensitively and in real time, consequently being /useful for estimation of the oxidative impacts to whole blood [9].
The present work aims to explore the effects of aspirin nanoparticles in rat blood on oxidative

stress through cyclic voltammetry (CV). In order to uncover the *in vivo* redox processes, post the administration of aspirin nanoparticles. This method is indicative for the investigation on the oxidative potential and biocompatibility of the nanoparticles of aspirin.

2. Materials and Methods

2.1 Chemicals and Reagent

Aspirin (analytical grade) were bought from Sigma-Aldrich. Phosphate-buffered saline (PBS), ethanol, and different reagents were of analytical grade and used without similarly purification. Deionized water became used for the duration of the experiments.

2.2 Preparation of aspirin nanoparticles

Lyophilization, or freeze-drying, is a broadly utilised approach in drug method that transforms quality aspirin powder into nanostructures thru the elimination of water via sublimation below low stress [10, 11]. Freeze-drying can enhance the formation of aspirin (ASP) nanoparticles by way of immobilising the active compound with cryoprotectants or suitable stabilisers, which includes mannitol or trehalose, at some stage in the freeze-drying process [12]. This method complements the drug's floor place, potentially growing its solubility and bioavailability, as illustrated by the Lyophilizer method [13] in Fig. 1

Fig 1: Lyophilization apparatus from LABCONCO Corporation (USA).

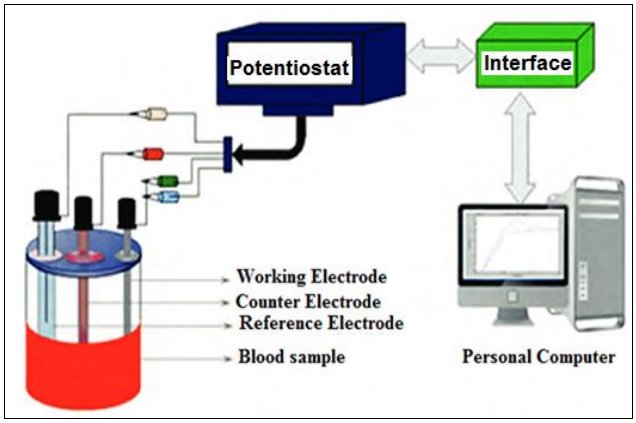
2.3 Animal Study Design

Twelve healthy male Wistar rats (200-250 g) were allocated into two groups: a control group (n = 6) administered saline, and a test group (n = 6) administered ASP nanoparticles (equal to 50 mg/kg aspirin) orally, as illustrated in scheme 1. After one month, the rats were anaesthetised, and blood was obtained via heart puncture into heparinised tubes. All experimental protocols received approval from the Institutional Animal Ethics Committee.

Scheme 1: Asp NPs doses the rat

2.4 Electrochemical Analysis

Cyclic voltammetry (CV) was conducted using an EZstat potentiostat/galvanostat from NuVant Systems Inc., USA. A standard three-electrode configuration was utilised, comprising a glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum wire counter electrode. The blood samples were diluted with deionised distilled water in a 1:9 ratio, and the potential was scanned from -0.2 V to +0.2 V at a scan rate of 100 mV/s. The electrodes were pre-treated with alumina slurry to guarantee a clean surface before to each measurement. All measurements were conducted at ambient temperature (25 °C). The configuration of the cyclic voltammetric system is depicted in Scheme 1. [14, 15]



Scheme 1: Setup of cyclic voltammetric technique

2.5 Field Emission Scanning Electron Microscopy (FESEM) study

ASP NPs were quantified using a particle dimension analyser in conjunction with a field emission scanning electron microscope (FESEM). Figure 2 illustrates the conversion of the dimension from 95 nm to 100 nm. ASP NPs at this dimension (97 nm) were employed to investigate the impact of nanoparticles on rodents after they were administered a concentration of... for one month. Subsequently, blood samples were analysed using electrochemical cyclic voltammetry.

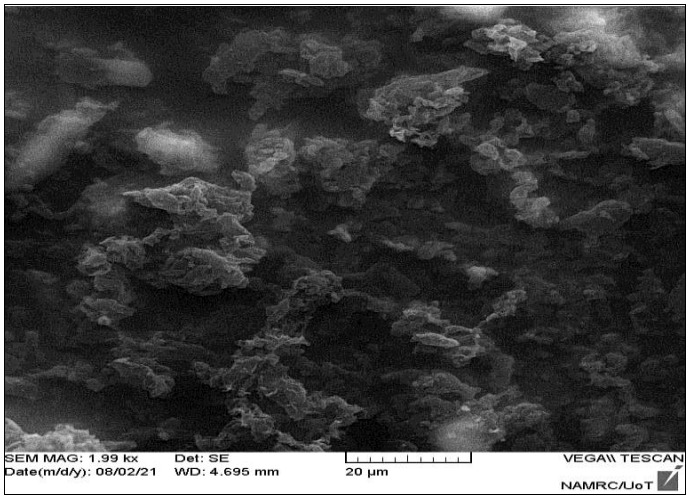


Fig 2: FESEM of ASP NPs

3 Findings and Analysis

3.1 Cyclic Voltammetry of Haematological Specimens

Figure 3 illustrates a such example CV curves. Control blood samples exhibited a basal redox behavior with a small redox peak at +0.5 V, which is consistent with endogenous redox-

active speciesinclude uric acid and ascorbic acid [16].in contrast, the ASP NPs treated group exhibited a prominent anodic peak at +0.45 V. The decrease in anodic peak current (Ipa) from 60 µA in the control group to 40 µA in the ASP NPs treated group indicates the effectiveness of the nanoparticles in regulating blood oxidation, which suggests a reduced effect of ASP NPs on blood oxidation. This shift in peak potential and decreased current intensity also indicate antioxidative stress and the potential interaction of ASP NPs with redox components in the blood. Cyclic voltage patterns show significant redox changes in the blood of mice after administration of aspirin nanoparticles. The observed decrease in anodic current and positive shift in peak voltage indicate decreased oxidative activity, This can be attributed to the interaction of aspirin nanoparticles with plasma proteins and cell membranes. [17].

Several mechanisms can explain the increased redox activity:

- ASP-NPs may induce mitochondrial antioxidant responses, leading to reduced oxidative stress [18].
- **Surface redox cycle:** The surface of nanoparticles can facilitate redox reactions, enhancing electron transfer processes in blood plasma [19].
- **Protein-nanoparticle interactions:** Adsorption of proteins to nanoparticle surfaces (protein crown formation) may alter the electrochemical properties of blood components [20,21] and cell membranes [17].

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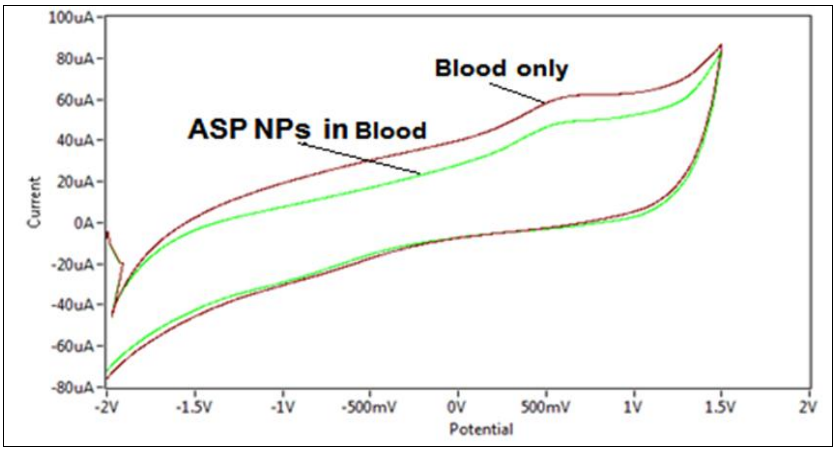

Fig 3: Presents the cyclic voltammogram of a blood sample and ASP nanoparticles (NPs) within the blood, utilising a glassy carbon electrode (GCE) as the working electrode and an Ag/AgCl reference electrode, with a scan rate of 0.1 V/s.

3.1 Impact of Varied pH Levels of ASP nanoparticles in rat blood samples

Figure 4 depicts the behaviour of ASP nanoparticles in rat blood samples across varying pH levels, as evidenced by cyclic voltammetry data. An oxidation peak current was observed in acidic pH (3-10), which vanished at a blood pH of 3 and intensified at an alkaline pH of 10; these characteristics are further depicted in Figure 5.

1. PH-Dependent Stability and Surface Charge of ASP NPs

ASP-NPs also display pH-sensitive characters thanks to the ionizable carboxyl group in aspartic acid. At physiological pH (approximately 7.4), the majority of carboxylates are deprotonated leading to an overall poor surface cost and colloidal balance in blood plasma. However, in pH-modifying environments together with modestly acidic ones (pH 6.0−6.5) corresponding to within inflamed tissues or tumor web-sites, protonation in the carboxylates decreases their surface area charge, producing them vulnerable to agglomeration and floating [22].

2. Protein Corona alterations and clearance

The protein corona developed on the surface of the ASP-NP particles in the blood sensitive to pH-associated surface chemistry. Mild acidification can also influence the affinity and population of adsorbed plasma proteins which change their interaction with macrophages, leading to greater clearance by the mononuclear phagocyte system. This is of particular relevance for the pharmacokinetics of ASP-NP where at low pH, conformational changes of ASP-NP surface proteins and corona proteins could uncover different isotopes which number larger would lead to faster clearance.

3. Effect of pH on Hemocompatibility and Toxicity

Acid-stimulated aggregation of ASP-NPs increases hemolysis and increases the risk of activation. When ASP-NPs enter compartments with slightly lower pH (such as ischemic or tumor microenvironments), this aggregation enhances their binding to plasma proteins and may affect red blood cell integrity. Preclinical models in mice have shown that ASP-NPs at pH 6.8 induce significantly greater hemolysis and inflammation markers compared to those at pH 7.4.

4. Biodistribution and Targeting Efficiency

While the reduced stability of colloids under acidic conditions can be problematic, it can also be exploited to enhance targeted delivery. ASP-NPs tend to destabilize in acidic microenvironments, facilitating payload release, especially when the pH is lower than in healthy tissues. A similar strategy has been demonstrated with polysuccinimide systems (a precursor to poly (aspartic acid)) in plants, where pH-catalyzed deconvolution improves site-specific payload delivery [23].

5. Rat Blood Model Reflects Translational Relevance

The murine blood model used here exhibits realistic circulatory responses, closely consistent with *in vivo* observations in larger mammals (including humans) regarding aggregation, hemolysis, and immune activation. The high bloodstream pH (6.5-6.8) resulted in significant uptake of ASP-NPs by macrophages in mice. *In vivo*, these conditions can occur transiently during ischemia, inflammation, or tumor metabolism.

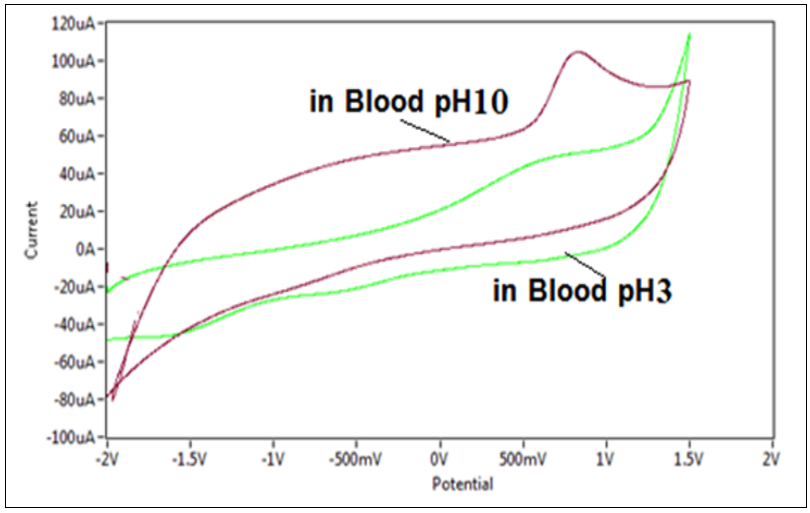

Fig 4: Cyclic voltammogram of ASP nanoparticles in a rat blood sample at varying pH levels, utilising a glassy carbon electrode as the working electrode and an Ag/AgCl reference electrode, with a scan rate of 0.1 V/s.

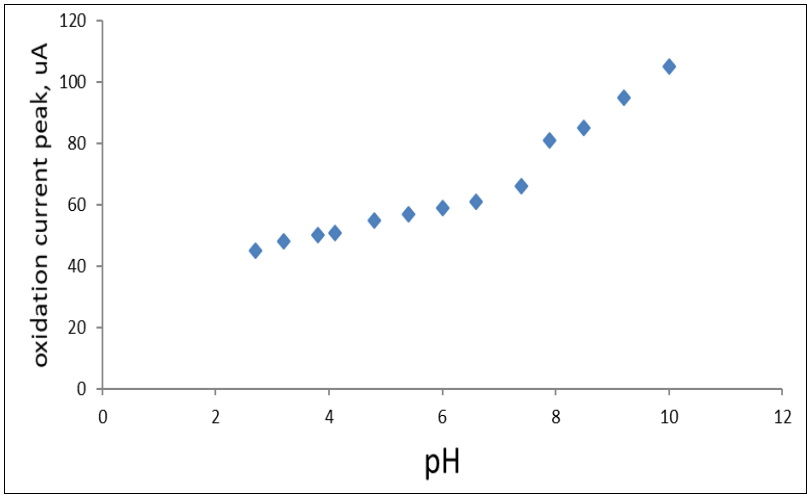

Fig 5: Correlation between the oxidation current peak of ASP nanoparticles in rat blood samples and varying pH levels (3-10)

3.2 Impact of Varying Temperatures on ASP Nanoparticles in Blood Samples

- This research examines the influence of temperature fluctuations on the electrochemical properties of ASP nanoparticles in rat blood samples. Analysing the temperature dependence of redox and diffusion properties is essential for enhancing the stability, storage, and pharmacokinetics of ASP nanoparticles. Cyclic voltammetry (CV) was employed to assess the electrochemical responses of the nanoparticles across a temperature range of 25 to 60°C.

1. Temperature-Dependent Protein Crown Formation

- Temperature significantly influences the structure and stability of the protein crown surrounding ASP nanoparticles. In typical nanoparticle systems (such as silver nanoparticles), researchers observed distinct crown patterns when incubated at 4, 17, 30, 41, and 47°C in plasma, despite constant pH. Some core proteins remained bound across all temperatures, but many proteins dynamically exchanged with temperature changes [24]. Furthermore, copper nanoparticles demonstrated an elevation in total protein adsorption as the temperature rose from 15°C to 42°C. It is reasonable to expect that ASP-NPs behave similarly—high temperatures are likely to promote broader uptake of opsonins, potentially accelerating clearance via phagocytosis by macrophages.

2. Colloidal Stability vs. Aggregation

- Temperature-induced aggregation is a major concern. In blood, ASP-NPs stabilized by albumin or other corona proteins can maintain their colloidal suspension at physiological temperature (37°C). However, deviations increase the risk:
- At lower temperatures (e.g., 4°C), decreased thermal mobility can promote aggregation, as demonstrated in accelerated aging studies of HSA-coated SPIONs
- At higher temperatures (above 37°C), corona restructuring can destabilize the suspensions or alter the

surface charge, enhancing aggregability [25].

3. Circulation Half-Life and Pharmacokinetics

- For thermosensitive polymeric nanoparticles, even small increases in temperature can significantly alter circulation times. For example, the half-life of thermosensitive liposomes decreased from approximately 2 hours at normal temperature to approximately 0.9 hours after heat exposure. Although this is not specific to ASP-NPs, it suggests that febrile conditions (38-40°C) in mice may significantly shorten the half-life of nanoparticles and alter their biodistribution patterns [26].

4. Blood Component Interactions and the Potential for Hemolysis

- Temperature fluctuations also directly affect blood components. At low temperatures, the rigidity of the red blood cell membrane increases, increasing the risk of hemolysis when interacting with ASP nanoparticles. High temperatures (over 40°C) may destabilize cells or alter protein interactions with nanoparticles, potentially activating complement or coagulation cascades [27].
- **Implications for *in vitro* and *in vivo* studies:** In standard In standard mouse blood incubation assays, It is imperative to sustain a consistent temperature of 37°C. Any deviation, even as small as ±5°C, may distort the results:
- Low temperatures may underestimate clearance and overestimate circulation time.
- High temperatures (e.g., during fever or induced hyperthermia) may overestimate the effects of clearance and aggregation, thus underestimating therapeutic efficacy.

3.3. Study on Reliability and Stability

- Performing a CV study on ASA NPs within a simulated rat blood model can yield significant insights into their reliability and stability, essential for biomedical applications, while ensuring that all findings are recorded and compared with prior studies for a thorough understanding [28]. In electrochemical investigations utilizing cyclic voltages, it is essential to validate the appropriate cyclic voltages through tests that yield a steady voltage, conducting 10 scans to identify voltage overlap for enhanced accuracy in the results. Figure 6 demonstrates the dependability of high voltage stability, facilitating consistent outcomes [29].

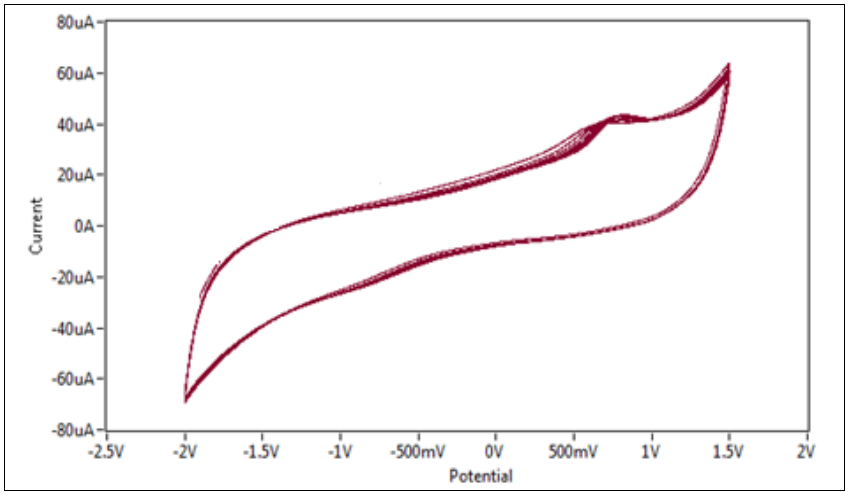

Fig 6: Cyclic voltammogram depicting ten scans of ASP nanoparticles in a rat blood sample on a glassy carbon electrode (GCE) against an Ag/AgCl reference electrode, with a scan rate of 0.1 V/s.

4. Conclusion

This research illustrates that aspirin nanoparticles provoke measurable oxidative changes in rat blood samples, as studied by cyclic voltammetry. These results highlight the importance of electrochemical methods for assessing nanoparticle-induced oxidative stress *in vivo* study. These electrochemical results were correlated with biochemical indicators of oxidative stress and tissue-level damage. The results demonstrated that ASP NPs induced antioxidant activity in rat blood, which in turn helps prevent the damage to gastrointestinal cells that occurs with pharmaceutical-grade aspirin.

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