doi.org/10.36721/PJPS.2023.36.5.SP.1583-1589.1

Formulation of aspirin nanoparticles using solvent evaporation method and *in vivo* evaluation of its antithrombotic effect

Lara Alhajj[1]*, Collins Ovenseri Airemwen[1,2] and Leyla Beba Pozharani[3]
[1]Faculty of Pharmacy, Cyprus International University, Nicosia, Cyprus
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin-City, Nigeria
[3]Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, Cyprus

Abstract: Nanotechnology offers several advantages in the treatment of chronic diseases through site-specific and target-oriented drug delivery. The purpose of this study was to formulate aspirin nanoparticles using the solvent evaporation method and evaluate the physicochemical properties of nanoparticles using X-ray diffraction (XRD), scanning electron microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and zeta sizer. *In vitro* dissolution studies, release kinetics, antioxidant and *in vivo* antithrombotic studies were also done. The formulated aspirin nanoparticles had a smooth and spherical shape with particle size of 76.25-128.17nm, polydispersity index of 0.34-0.46 and encapsulation efficiency of 36.29-42.52%. The nanoparticles were positively charged and had a zeta potential value of ≤+47.64 mV. The *in vitro* release studies showed that the aspirin nanoparticles released the drug in a sustained release manner simulating the Higuchi release model via non-Fickian diffusion mechanism. The formulated aspirin nanoparticles also showed significant antioxidant and antithrombotic effects in experimental rats ($P<0.05$). Aspirin nanoparticles were formulated in this study which may offer better therapeutic advantages over the conventional aspirin tablets in the prevention and management of acute myocardial infarction and ischaemic stroke.

Keywords: Aspirin, nanoparticles, myocardial infarction, thrombosis.

INTRODUCTION

The use of nanotechnology in the manufacture of pharmaceutical products is altering the scientific approach to disease prophylaxis, diagnosis and therapy by offering several advantages in the treatment of chronic human diseases through site-specific and targeted drug delivery (Narayan *et al.,* 2018, Bayda *et al.,* 2019). Various nanopharmaceuticals formulated as nanotechnology-based systems such as carbon nanotubes, liposomes, polymeric nanoparticles and dendrimers have resulted in significant improvement in drug delivery as well as the overall medical sciences (Aminu *et al.,* 2020, Ibrahim *et al.,* 2021).

The unique qualities of nanoparticles such as their high surface area, flexible surface chemistry and small sizes enable them to overcome many of the limitations of conventional drug delivery methods, such as poor solubility, low bioavailability and off-target effects (Jaafar *et al.,* 2023). Formulating drugs in nanoscale increases the surface to volume ratio leading to a higher dissolution rate of the drug and enhanced bioavailability (Rizvi and Gutkin, 2019).

Nanoparticles (NPs) are the most important part of nanomedicines and there are several types of nanoparticles based on their shapes, such as rods, wires, spheres, multipodes, stars, sheets and cages (Halwani, 2022). These particles can carry and deliver drugs as well as agents used for diagnostic imaging and sensing to the specific organs and tissues (Munawar *et al.,* 2019; Airemwen and Halilu, 2022).

Nanopharmaceuticals can be used to treat several diseases such as diabetes, hypertension, neurological disorders and cancer (Prasad *et al.,* 2018). They can also be used to improve the delivery of existing drugs, increase their effectiveness and reduce the risk of side effects (Airemwen and Obarisiagbon, 2023). Additionally, nanopharmaceuticals can be used to deliver diagnostic agents, aiding earlier detection and prophylaxis of diseases (Wadhwa *et al.,* 2018). Also, different drugs can be delivered to specific targets using sophisticated passive or active targeting techniques. Molecular recognition methods can also be used to direct the nanocarrier to specific cells or tissues by attaching different ligands or targeting agents to its surface (Mizrahy *et al.,* 2019; Majumder and Minko, 2021).

Aspirin is a cost-effective non-steroidal anti-inflammatory drug (NSAID) often used for the treatment of fever, pain and inflammation hence it is an analgesic, antipyretic and anti-inflammatory drug (Fuster *et al.,* 1998). It is also an anti-platelet drug used in the prevention of coagulopathy in Covid-19 patients and thrombotic disorders. Previous studies have reported that aspirin can be used in the treatment of the significant increase in platelet aggregation and neutrophil in post covid patients (Marcus, 1983; Aminu *et al.,* 2020). Aspirin acts by inhibiting the cyclooxygenase (COX) enzyme, which is involved in the

**Corresponding author:* e-mail: lalhajj@ciu.edu.tr

conversion of arachidonic acid to thromboxane A_2 which is a platelet agonist that induces enhanced platelet aggregation in response to platelet activators. Cyclooxygenase enzyme cannot by synthesized by platelets once inhibited, hence thromboxane recovery is dependent on the formation of new platelet at a slow rate of 15% per day (Wallace *et al.*, 1997). Aspirin has also been reported to exhibit saturation pharmacokinetics, which means that when it binds to clots, it causes them to become saturated, resulting in their deactivation. Thromboembolic disease and acute myocardial infarction (AMI) and are a major cause of hospitalization and mortality globally, hence the formulation of effective drugs for the prophylaxis and treatment of these disease conditions has been of great scientific interest. Acetylsalicylic acid (ASA) has been previously reported to possess significant anti-platelet aggregation function and it had been used extensively for the prevention and therapeutic management of AMI and thrombosis. Previous scientific research has shown that ASA can lower the incidence rate of peripheral and cardiovascular thromboembolic diseases by 25% (Miyake *et al.*, 2015).

Previous studies have also shown that chronic use of low-dose acetylsalicylic acid (ASA) in the treatment of stroke and myocardial infarction significantly improves the therapeutic outcome and reduce the incidence of these disorders thereby improving the quality of life of the patients. However, oral administration of ASA tablets is known to cause gastric ulcer and bleeding as side effects and some patients may develop aspirin failure (AF) or aspirin resistance (AR) (Weber *et al.*, 2002). Aspirin also has stability issues which affect the pharmacokinetic parameters of the drug and it also undergoes hydrolysis rapidly to salicylic acid and this has been a major challenge in its formulation as a liquid dosage form such as injection (Luo *et al.*, 2018). These are some of the limitations of oral ASA dosage forms and formulation of aspirin nanoparticles will significantly reduce these side effects, improve the pharmacological properties of ASA as well as improve its therapeutic benefits in patients with acute ischaemic attack or stroke (Miyake *et al.*, 2015). Hence the purpose of this study was to formulate aspirin nanoparticles and to evaluate their particle sizes, encapsulation efficiency, *in vitro* drug release kinetics and antithrombotic effects.

MATERIALS AND METHODS

Materials

Acetylsalicylic acid (Cipla Limited, India) was the active pharmaceutical ingredient used in this study, 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma Aldrich, Germany), Eudragit RL 100 (Rhoma Pharma, Germany), Sodium Alginate (Sigma, Germany) Dichloromethane (Emsure, India), Polyvinylpyrrolidone (PVP, Ranbaxy, India). The other chemicals used in the study were of analytical grade.

Adult male and female rats weighing (250-300g) were purchased and housed at the Animal House Facility of the Faculty of Pharmacy, University of Benin, Nigeria. Animals were kept under good housing conditions and allowed to adapt for two weeks before the experiments started. The Faculty of Pharmacy Ethics Committee, University of Benin, Nigeria granted the Ethical approval with Reference No: EC/FP/016/24. All animal experiments were done according to standard procedures previously reported by Airemwen *et al.* (2021).

Methods

Formulation of aspirin nanoparticles

Aspirin nanoparticles were prepared using the solvent evaporation method (Chourasiya *et al.*, 2021). Sodium alginate (0.01 g) was weighed and dissolved in 25 mL of dichloromethane (DCM) and stirred continuously using a magnetic stirrer to form a colloidal solution (organic phase). Polyvinylpyrrolidone (0.2%) was added as the stabilizer and the internal phase. Aspirin (100 mg) was weighed and added into the organic phase and stirred continuously to obtain a homogenous solution. Both the internal and organic phases were homogenized to form the primary emulsion which was then sonicated for 5 min. The organic solvent was allowed to evaporate overnight for 15h. Finally, the aspirin nanoparticles were lyophilized using a freeze dryer and collected. Three batches of the aspirin nanoparticles were formulated (table 1).

Evaluation of the formulated nanoparticles

Scanning electron microscopy (SEM)

The shape and size of the nanoparticles were examined using a SEM (Shimadzu, Japan).

Polydispersity index (pdi), particle size and zeta potential

PDI, particle size distribution and zeta potential were examined using a zeta sizer (Mavern Instrument, UK).

Drug entrapment

The aspirin nanoparticles (10 mg) was mixed with 5 mL of alcohol and sonicated using a sonicator (Misonix, USA) for 30 min. It was then centrifuged for 5 min at 2000 rpm. The amount of aspirin in the supernatant layer was analyzed with the use of UV equipment (Shimadzu, Japan). Drug entrapment (E) of the formulations was calculated using Equation 1.

$$E = \frac{\text{Total drug in the formulation-Drug in the supernatant}}{\text{Total drug in the formulation}} \times 100$$

Fourier transform ınfra-red spectroscopy (FTIR)

The drug-excipient compatibility of the formulated nanoparticles was evaluated using FTIR spectrometer (Shimadzu, Japan).

X-ray Diffraction (XRD) Analysis

The optimized nanoparticle was done analyzed using Rikagu generator (XRD Rikagu Rint 2000, Japan).

Evaluation of antioxidant activity of the nanoparticles using DPPH

The pure aspirin and the nanoparticles (10 mg) each were used to prepare a 10 mg/mL stock solution. Serial dilutions were done to obtain 5, 2.5, 1.25 and 0.25 ug/mL solutions. The DPPH (0.8 mM) was prepared in methanol and then added to the test tubes containing the samples and stored in the dark for 1 h. The UV spectrophotometer was used to measure the absorbance of each sample at 450 nm. Percentage inhibition was calculated using equation 2.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}}$$

In vivo evaluation of carotid artery thrombosis in rats

This experiment was done by method previously described by Luo *et al.*, 2018 with some modification. Arterial thrombosis was formed by stimulating the carotid artery of the experimental rats electrically. The rats were anaesthetized intraperitoneally at a dose of 25 mg/kg. The carotid artery of the rats was isolated and attached to the stimulation electrode of an experimental thrombosis detector (BT87-3, China), (Qin *et al.*, 2005). Five (5) healthy male or female Wister rats assigned randomly to four (4) groups: pure aspirin group (0.5 g/kg), aspirin nanoparticle group (0.25 g/kg), aspirin nanoparticle group (0.5 g/kg) and control group. The drug in each group was administered to the rats by gavage and the carotid artery of the rats was stimulated electrically after 30 min of drug administration at an intensity of 2.0 mA for 10 min in order to damage the endothelial cells thereby resulting in gradual formation of blood clot in the blood vessel (Yu *et al.*, 2001). The flow of blood through the carotid artery was occluded by the clot causing a decrease in the temperature of the carotid artery which triggered the alarm of the equipment. The time period between the start of blood vessel stimulation to the sudden decrease in the carotid artery temperature is called the Occlusion Time (OT) and it was recorded by the thrombosis detector.

In vitro drug release studies

The release study of aspirin from the formulated nanoparticles was done by method previously described by Airemwen and Halilu, 2022.

In vitro kinetics

The data gotten from the drug release studies were analyzed using the zero, first order, Higuchi and Korsemeyer-Peppas release models in order to obtain the release kinetics.

STATISTICAL ANALYSIS

All tests were done in triplicate. The mean and standard deviations (SD) values were calculated. Statistical analyses were done using Microsoft excel and SPSS 29.0.

RESULTS

Morphology of aspirin nanoparticles

The scanning electron microscope (SEM) image of the optimized aspirin nanoparticles is shown in fig. 1 and it showed that the nanoparticles had a spherical, porous and crystalline structure.

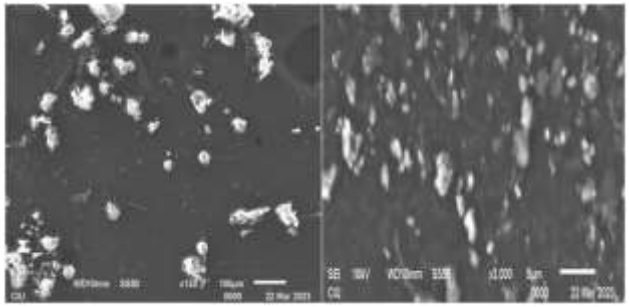

Fig. 1: SEM image of optimized aspirin nanoparticles.

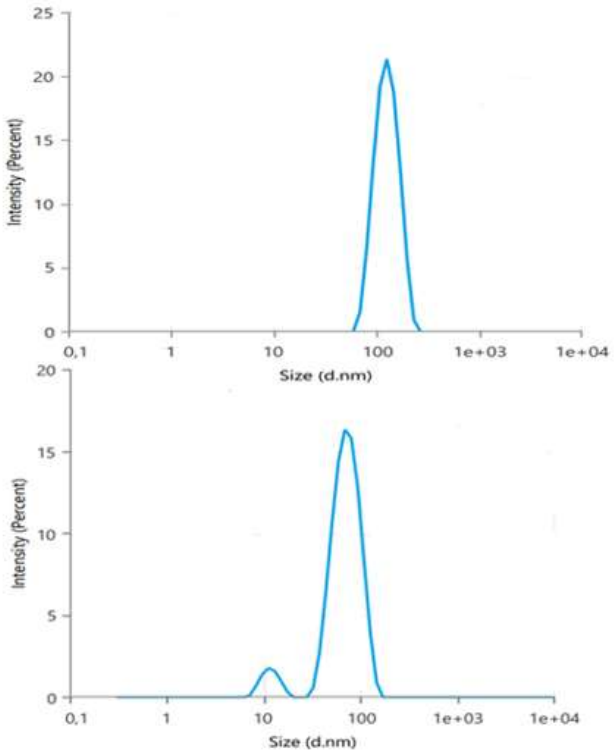

Fig. 2: Particle size distribution of aspirin nanoparticles

PDI, particle size, zeta potential and entrapment efficiency

Table 2 shows the results of the PDI, particle size, zeta potential and entrapment efficiency and fig. 2 shows the particle size distribution. The PDI is an important parameter that explains the range of the distribution of particle sizes. The PDI values of all the formulations were ≤0.46±0.11. Batches AN1, AN2 and AN3 had particle sizes of 76.25±0.01 nm, 94.02±0.01 nm and 128.17±0.01 nm respectively. Zeta potential values of ≤+47.64± 0.01mV. Batches AN1, AN2 and AN3 had entrapment

efficiency values of 36.69±0.11, 44.37±0.14 and 42.52±0.10% respectively. Batch AN2 had the highest entrapment efficiency value among the formulated nanoparticles. These results were similar to the findings of previous studies done by Luo *et al.* (2018).

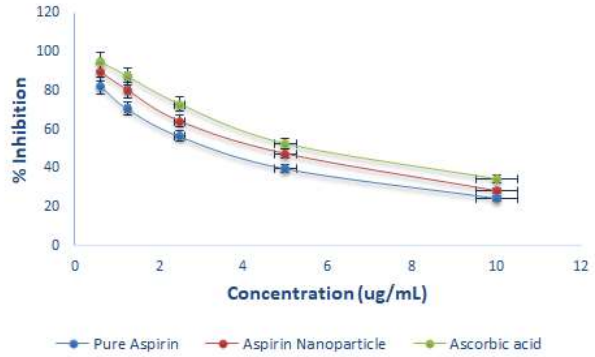

Fig. 3: Percentage inhibition of pure aspirin, aspirin nanoparticles and ascorbic acid on DPPH.

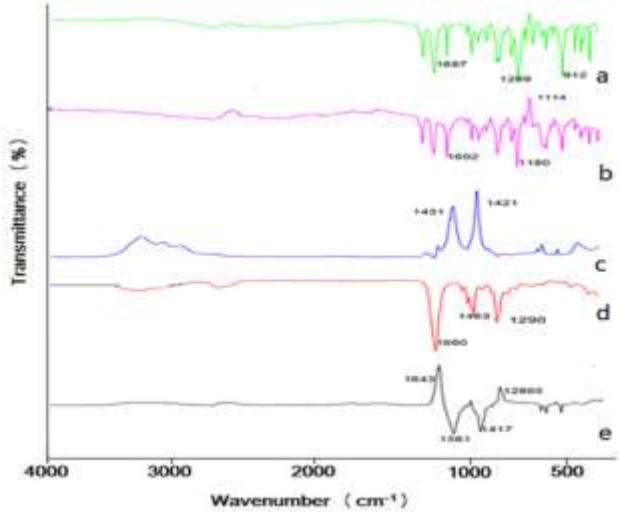

Fig. 4: FTIR spectra of (a): pure aspirin (b) aspirin loaded nanoparticles (c) sodium alginate (d) dichloromethane (e) polyvinylpyrrolidone.

Antioxidant activity

DPPH scavenging activity of aspirin nanoparticles

The results of the antioxidant assay were expressed as IC_{50} and percentage inhibition and revealed that the pure nanoparticles had a higher antioxidant activity than the pure aspirin at all concentrations. Although, the ascorbic acid showed a higher antioxidant activity than both pure aspirin and the optimized aspirin nanoparticles. However, there was no significant difference between the antioxidant effects of pure aspirin sample, optimized aspirin nanoparticles and ascorbic acid ($P>0.05$). From the graph of the percentage inhibition against the concentration, the IC_{50} of the pure aspirin, optimized aspirin nanoparticles and ascorbic acid were 3.10, 4.10 and 4.90 ug/mL respectively (Table 1 and Fig. 6). The results obtained were similar to findings of previous studies done by Pavithra and Sasikumar (2015).

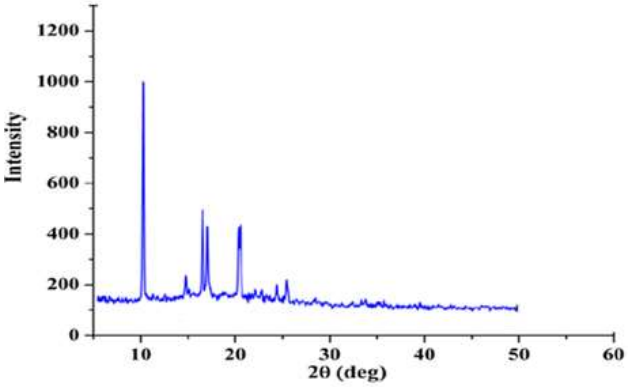

Fig. 5: XRD spectrum of optimized aspirin nanoparticle.

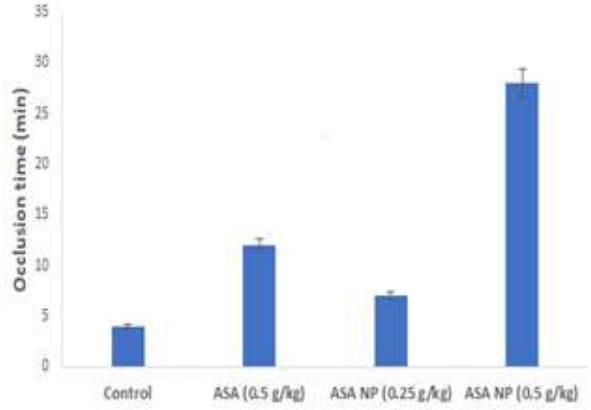

Fig. 6: Effect of control, aspirin pure sample (0.5g/kg) and aspirin nanoparticles (0.25g/kg and 0.5g/kg) on carotid artery thrombosis in rats.

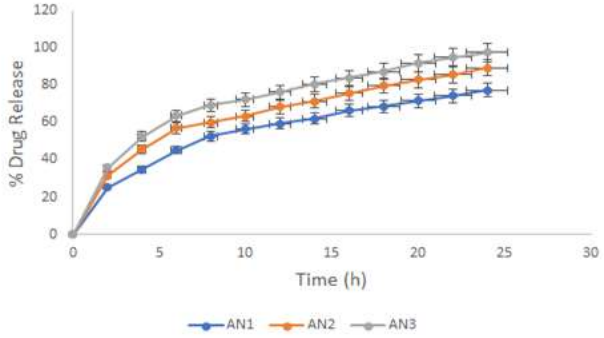

Fig. 7: *In vitro* drug release profile of the formulated nanoparticles.

Fourier transform infrared spectroscopy analysis

The physical stability and the drug-excipient compatibility were analyzed using FTIR spectroscopy. The pure aspirin showed characteristic peaks at 1687cm^{-1} (O-H stretching-hydroxy group), 1289cm^{-1} (C=O stretching- carboxylic acid) and 912cm^{-1} (C-O stretching-ester) while the optimized nanoparticle formulation showed characteristic peaks at 1602cm^{-1}, 1180cm^{-1} and 1114cm^{-1}. There was no significant change in the peaks of

Table 1: Formula of aspirin nanoparticles

Batches	Aspirin (mg)	Sodium alginate (g)	PVP (%)	DCM (mL)
AN1	100	0.01	0.2	25
AN2	150	0.02	0.2	30
AN3	200	0.04	0.2	35

Table 2: PDI, particle size, zeta potential and entrapment efficiency results of the aspirin nanoparticles

Formulation	PDI ± SD	Particle size (nm) ± SD	Zeta potential (mV) ± SD	EE (%) ± SD
AN1	0.34 ± 0.02	76.25 ± 0.01	+36.28 ± 0.02	32.69±0.11
AN2	0.37 ± 0.01	94.02 ± 0.01	+39.53 ± 0.01	44.37±0.14
AN3	0.46 ± 0.11	128.17 ± 0.01	+47.64 ± 0.01	42.52±0.10

Table 3: Percentage inhibition of aspirin, optimized aspirin nanoparticles and ascorbic acid against DPPH

Conc. (ug/mL)	Pure aspirin (%)	Aspirin nanoparticles (%)	Ascorbic (standard) (%)
10	24.58	28.54	34.54
5	39.86	47.61	52.68
2.5	56.48	64.26	72.95
1.25	70.91	80.43	87.41
0.625	82.46	89.42	94.69

Table 4: Release kinetics of different batches of aspirin nanoparticle formulations

Models	Zero		First		Higuchi		Korsmeyer and Peppas	
Formulations	r^2	K_0	r^2	K_1	r^2	K_H	r^2	n
AN1	0.855	2.64	0.969	-0.024	0.986	15.49	0.720	0.66
AN2	0.834	2.92	0.977	-0.035	0.979	17.29	0.685	0.72
AN3	0.810	3.15	0.944	-0.056	0.970	18.85	0.670	0.75

both the pure aspirin and the formulated nanoparticles hence it can be deduced from the study that the aspirin and the excipients used in the formulation were compatible. This result is similar to previous studies done by Nagaraja *et al.,* 2020.

XRD analysis

XRD is an analytical method that provides detailed information about the polymorphic, crystallographic structure, chemical composition, and physical properties of drugs. XRD analysis was conducted to determine the crystalline structure of the formulated nanoparticles. The XRD spectra showed that the Bragg's reflection peaks for the optimized aspirin nanoparticles were found at 2 theta of 10°, 15°, 16°, 18°, 21°, 25° and 27° which correspond to 1000, 220, 500, 470, 490, 180 and 205 lattice planes respectively. Similar results were obtained by Ozturk *et al.,* 2019. The X-ray diffractions were recorded between an angle of 20° and 80°. The highest intensity peak was seen at the (1000) plane.

Effect of aspirin nanoparticles on carotid artery thrombosis in rats

The results showed that aspirin nanoparticles at a dose of 0.25 g/Kg had no significant effect on prolonging the OT of carotid artery thrombosis compared with the control group but at a dose of 0.5g/Kg, aspirin nanoparticles had significant effects which increased as the dose of the drug was increased (*P<0.05). The results showed that aspirin nanoparticles prevented the carotid artery thrombosis in a dose-dependent manner. At a dose of 0.5 g/Kg, the aspirin nanoparticles had significant effects on prolonging the OT of carotid artery thrombosis compared with the control group and 0.5 g/Kg pure aspirin. This may be due to the reduced particle size and increased surface area of the nanoparticles compared to pure aspirin, Similar findings were also observed by Luo *et al.,* 2018.

In vitro drug release kinetics

Fig. 4 shows the results of the *in vitro* drug release studies of the formulated aspirin nanoparticles. It was observed from the result that 25.23%, 31.48% and 35.82% of aspirin was released from batches AN1, AN2 and AN3 respectively in 2 h followed by a prolonged release of drug over a 24 h duration. Previous studies by Chourasiya *et al.,* 2021 obtained similar results.

DISCUSSION

The formulated aspirin nanoparticles had a smooth and porous crystalline structure with particle size values between 76.25-128.17 nm, PDI values were ≤0.46±0.11 and zeta potential values between +36.28 - +47.64 mV. The surface morphology of nanoparticles is an important

characteristic that influences the drug absorbance properties and release. PDI value is used to evaluate particle size distribution and it is between 0.01 and 0.7 for single phase systems. A value greater than 0.7 indicates heterogeneous distribution. PDI value of all the formulated nanoparticles in this study was determined to be ≤0.46±0.11 which indicates homogenous particle distribution. Stearic and electrostatic charges determine the stability of nanoparticles dispersed in aqueous media and a high zeta potential value indicates good colloidal dispersion stability. Hence, the formulated nanoparticles were stable (Airemwen and Halilu, 2022). These results are similar to previous studies done by Ozturk *et al.,* 2019 who formulated dexketoprofen trometamol loaded nanoparticles. The FTIR results showed there were no significant changes in the peaks of the optimized aspirin nanoparticles and pure aspirin which shows that there was no chemical interaction between aspirin and the excipients used in the preparation of the nanoparticles and this shows that the formulation was stable and compatible.

The XRD analysis was performed to evaluate the crystallinity and polymorphic characteristics of the formulated nanoparticles. The results of the XRD study revealed the aspirin nanoparticles existed in the crystalline and stable polymorphic form. This result is also similar to previous studies done by Ozturk *et al.*, 2019.

The aspirin nanoparticles at a dose of 0.5 g/kg significantly delayed the OT of carotid artery thrombosis compared with the control group and 0.5 g/Kg aspirin hence they possessed significant antithrombotic and antiplatelet effects (*P<0.05). Previous studies done by Luo *et al.,* 2018 also obtained similar results and the increased antithrombotic effect of aspirin nanoparticles may be due to the fact that they possess a higher surface area and smaller particle sizes compared to the pure aspirin hence the increased activity.

The drug release studies revealed an initial onset of drug release from the formulated aspirin nanoparticles and this may be due to the accelerated dissolution of the adsorbed aspirin on the surface of the nanoparticles while the aspirin enmeshed in the polymeric matrix of the nanoparticles was released steadily over a long period of time (sustained drug release). This result is similar to previous studies done by Chourasiya *et al.* (2021). Formulation AN3 released the highest amount of aspirin in 24 h with a drug release of 97.64% compared to formulation AN1 with a drug release rate of 77.49% as shown in Fig. 4. From the results of the release kinetics and correlation coefficient studies, drug release mechanism from the nanoparticles simulated the Higuchi release model ($r^2 = 0.986$) which indicates that the aspirin was uniformly dispersed within the nanoparticles and the drug release kinetics from the formulations was diffusion controlled (Airemwen *et al.,* 2021). Analysis of the Korsmeyer-Peppas diffusion release model (n>0.5) suggest that drug release mechanism was via non-Fickian diffusion (Higuchi, 1963; Korsemeyer *et al.*, 1983).

CONCLUSION

Aspirin nanoparticles were formulated in this study using the solvent evaporation method. The formulated nanoparticles possessed significant antioxidant and antithrombotic effects (P<0.05) and this can be exploited after further studies to improve the therapeutic outcome of patients with acute myocardial infarction and ischaemic stroke.

REFERENCES

Airemwen CO, Isesele JE, Uchendu AP, Halilu ME and Uhumwangho MU (2021). Formulation of subcutaneous implantable drug delivery system of ibuprofen using biodegradable polymer. *Nig. J. Pharm. App. Sci. Res.*, **10**(4): 14-21.

Airemwen CO and Halilu ME (2022), Formulation and *in vitro* evaluation of polymeric metronidazole nanoparticles. *Pak. J. Pharm. Sci.*, **35**(5): 1333-1338.

Airemwen CO and Obarisiagbon AJ (2023). Formulation of silver nanoparticles from the leaves extract of *Vernonia amygdalina. Nig J. Pharm.*, **57**(1): 459-466.

Aminu N, Bello I, Umar NM, Tanko N, Aminu A and Audu M M (2020). The influence of nanoparticulate drug delivery systems in drug therapy. *J. Drug Del. Sci. Tech.*, **60**(1): 101-113.

Bayda S, Adeel M, Tuccinardi T, Cordani M and Rizzolio F (2019). The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. *Molecules*, **25**(1): 112-126.

Chourasiya V, Bohrey S and Pandey A (2021). Formulation, optimization and characterization of amlodipine besylate loaded polymeric nanoparticles. *Poly. Poly. Comp.*, **29**(9): 1555-1568.

Fuster V, Cohen M and Chesebro JH (1998). Usefulness of aspirin for coronary artery disease. *Am. J. Cardiol.*, **61**(2): 637-640.

Halwani AA (2022). Development of pharmaceutical nanomedicines: From the bench to the market. *Pharmaceutics.*, **14**(1): 106-112.

Higuchi T (1963). Mechanism of sustained action medication. Theoretical analysis of rate release of solid drugs dispersed in solid matrices. *J. Pharm. Sci.*, **52**(4): 1145-1149.

Ibrahim MA and Abdellatif AA (2021). Applications of nanopharmaceuticals in delivery and targeting. *Nanopharm: Principles App.*, **1**(1): 73-79.

Jaafar HJ (2023). The importance of nano pharmaceuticals and nanomedicines applications and Properties: A current review. *Technium Bio Chem Med.*, **5**(2): 1-29.

Korsmeyer RW, Gurny R, Doelker E, Buri P and Peppas NA (1983). Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.,* **15**(1): 25-35.

Luo S, Man H, Jia X, Li Y, Pan A, Zhang X and Song Y (2018). Preparation and characterization of acetylsalicylic acid/ chitosan nanoparticles and its antithrombotic effects, *Designed Monomers Poly*, **21**(1): 172-181.

Majumder J and Minko T (2021). Multifunctional and stimuli-responsive nanocarriers for targeted therapeutic delivery. *Expert opinion on drug del.*, **18**(2): 205-227.

Marcus AJ (1983). Aspirin as an antithrombotic medication. *Nat. Eng. J Med.*, **309**(2):1515-1517.

Miyake K, Teppei A and Yuriko H (2015). Proton pump inhibitors are associated with lower gastrointestinal tract bleeding in low-dose aspirin users with is chaemic heart disease. *Dig Liver Dis.*, **47**(1): 757-766.

Munawar A, Ong Y, Schirhagi R, Tahir MA, Khan WS and Bajwa SZ (2019). Nanosensors for diagnosis with optical, electric and mechanical transducers, *Royal. Soc. Chem. Adv.*, **9**(2): 6793-6803.

Nagaraja S, Kuldeep R, Muktika T, Dnyaneshwar K, Anroop BN, Katharigatta NV and Rakesh KT (2020). Development of metronidazole loaded chitosan nanoparticles using QbD approach-A novel and potential antibacterial formulation. *Pharmaceutics*, **12**(3): 920-928.

Narayan R, Pednekar A, Bhuyan D, Gowda C and Nayak UY (2017). A top-down technique to improve the solubility and bioavailability of aceclofenac: *In vitro* and *in vivo* studies. *Int. J. Nanomed.*, **12**(1): 4921-4935.

Ozturk AA, Yenilmez E and Yazan Y (2019). Dexketoprofen trometamol loaded Eudragit® RL 100 nanoparticle formulation, characterization and release kinetics, *Acta Pharm. Sci.*, **57**(1): 69-84.

Pavithra K and Sasikumar V (2015). Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima* (Jacq.) Cogn. *Food Sci. Hum Wellness.*, **4**(2): 42-46.

Prasad M, Lambe UP, Brar B, Shah I, Manimegalai J, Ranjan K, Rao R, Kumar S, Mahant S and Khurana SK (2018). Nanotherapeutics: An insight into healthcare and multi-dimensional applications in medical sector of the modern world. *Biomed. Pharmacother.*, **97**(1): 1521-1537.

Qin JM, Zhang YM and Yang YM (2005). Skills using model BT87-3 experimental intracorporeal thrombosis surveyor and precautions. *Chin. Pharm. Bull.*, **21**(1): 756-759.

Rizvi SA and Saleh AM (2018) Applications of nanoparticle systems in drug delivery technology. *Saudi Pharm J.*, **26**(1): 64-70.

Robless P, Mikhailidis DP and Stansby G (2001). Systematic review of antiplatelet therapy for the prevention of myocardial infarction, stroke or vascular death in patients with peripheral vascular disease. *British J. Surg.*, **88**(2): 787-800.

Mizrahy S, Gutkin A, Decuzzi P and Peer D (2019), Targeting central nervous system pathologies with nanomedicines. *J. Drug Target.*, **27**(1): 542-554.

Wadhwa A, Mathura V and Lewis S (2018). Emerging novel Nano pharmaceuticals for drug delivery. *Asian J. Pharm. Clin. Res.*, **11**(2): 35-42.

Wallace JL, Elliott SN and Del Soldato P (1997). Gastrointestinal sparing anti-inflammatory drugs: The development of nitric oxide-releasing NSAIDs. *Drug Dev Res.*, **42**(4): 144-149.

Weber AA, Przytulski B and Schanz A (2002). Towards a definition of aspirin resistance: A typological approach. *Platelets*, **13**(1): 37-40.

Yu FJ, Wei YL and Ding QH (2001). Experiment of arterial thrombosis by direct electric current stimulation. *Chin. J. Phys. Med. Retabulitation*, **1**(1): 41-42.