RESEARCH ARTICLE

Pharmacokinetic Study of Aloin Nanoparticulate: Enhanced Oral Formulation Bioavailability

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ABSTRACT

This research aimed to prepare and assess an aloin nanoparticle that might increase its oral bioavailability. Utilizing the nanoprecipitation process, aloin Poly lactic-co-glycolic acid (PLGA) nanoparticulate was formulated and studied for the best strategy based on their characteristics and *in-vitro* release. The aloin nanoparticle stability research was evaluated at room temperature for a duration of three months. Additionally, pharmacokinetic (PK) tests were analyzed by using various parameters and experiments done by taking female wistar albino rats, with weights of approximately (150–200 gm). The animal was appropriately given the proper housing according to the protocol and placed in a period of twelve hours day and night. The drug was administered for 72 hours at a dosage of 10 mg/kg. of all the aloin nanoformulations developed, the one with the best size of particles was 98.5 nm with an entanglement efficacy of 98.09%. The results show that when compared to pure aloin, aloin PLGA nanoparticulate has a higher bioavailability.

Keywords: Pharmacokinetic, Aloin, Bioavailability, *In-vivo*, Oral exposure, Validation method.

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INTRODUCTION

Aloin is a bioactive component in aloe, commonly used for nutrition, anticancer properties, and other activities. Simply it was a mixture of diastereomers having 2-isomers of aloin, as mentioned one isomer was aloin A and another isomer of aloin was aloin B, it is isolated from *Aloe barbadensis* related to the family called Liliaceae.[1,2] It is generally utilized in cosmetics, food items,[3] cancer, antidiabetics, antifungals, aromatics, antioxidants, heart stimulators, medicines, and therapeutic qualities, including laxative effects, as discussed by Koch in 1996.[4,5] It also has antibacterial and medicinal properties. Despite anthrone derivatives, chromones, and polysaccharides, anthraquinone is a physiologically active compound in aloe, as reported in 2004 by Tom. Aloe products were used to treat wounds and inflammation as per Park's report in 2005, but nothing is known about its pharmacokinetics investigation. Aloin according to research, aloin was found to be cytotoxic to cell lines for human breast cancer studied by Esmat in 2006[6] and on rats also in 1996 by Chung.[7] It also boosted the alcohol oxidation rate and prevented the production of AOM-induced DNA adducts in rats.[8] To better understand the advantages and risks of aloin, research the oral studies.

MATERIALS AND METHODS

Materials

Aloin as a drug, Poly lactic-co-glycolic acid (PLGA) as a polymer, Milli-Q Plus H_20, double-distilled water (H_2O) (Millipore, USA), and polyvinyl alcohol as a stabilizer were used as chemicals.

Aloin Nanoformulation Preparation

Using a lab scale, the nanoparticulate is produced by using the nanoprecipitation process at room (temp) temperature.[9,10] Drug (Aloin), polyvinyl alcohol, and PLGA were all added to the formulation while it was continually stirred.[11,12] The nanoformulation aloin preparation parameters, including the polymer concentration, time and concentrated stabilizers were optimized according to the method of central composite.[13,14]

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Particle size as observed in nm and entangled effectiveness in % were evaluated as two dependent variables of the drug contents show in Table 1.[15]

Drug Stability Study[16]

According to ICH criteria, optimized nanoformulation of drug aloin stabilized was tested for three months at different ranges of humidity, temperature taken 25 2°C 60% taken as relative humidity (RH), and 400° 2°C and 70.5% RH. The optimized nanoparticle's zeta potential (ζ), (nm) particle size and degree of entrapment (%) were all examined.[17]

Bioavailability Study[18] (Experimental Animals)

Formulations that were employed in this study: (1) Aloin suspension; and (2) Aloin nanoparticle. According to institutional policies and with the agreement of ethical authorities, the *in-vivo* pharmacokinetic (PK) research of the aloin drug nanoparticle (Np) was carried out.[19] Before beginning the research, an experimental procedure was authorized by the Institutional Animal Ethical Committee (1335/PO/Re/S/10/CPCSEA); protocol number MMCP-IAEC-93.

Healthy adult albinos were used to differentiate the nanoformulation examination of the drug's kinetic characteristics in plasma from the formulation of the standard medication.[20] Wistar rats taken with the approx. body wt. 150 to 200 g was employed within the investigation.

Experiments were conducted with the current guidelines of the CPCSEA's. Evaluation of drug kinetic parameters in blood/ plasma was done by using nanoformulation differentiated from the formulation of the normal drug by using healthy rats weighing between 150 and 200 g and category adult albino Wistar was taken for the study.[21]

Animal Treatment

A week previous to the experiment, rats were kept in (PP) polypropylene housing/cages with twelve hours of light (day) and dark cycles (night), an ambient temp., and a relative humidity of 40–70%.[22,23] Animals were given water, *ad libitum*, and rat food.

Three groups of 6 (six) rats were created each single group formed at least 6 animals.

The 0.9% solution (sol.) of saline used in group I served as the control.

Pure aloin in water for injection in group II

Nanoformulation of aloin; group III.

The procedure used was injecting the pre-sterilized samples intravenously at 10 mg/kg dosage through a tail vein. About 200 µL of blood/plasma samples were drawn in Eppendorf tubes from the retro-orbital cavity of the animal with periods of different hours 0, 0.25 hours, then taken samples 0.5 hour, afterword 1-hour, then 2 hours, then 4 hours, then collected within 8 hours, and next sample collect within 12 hours, then 24 hours, after one day 48 hours, and finally 72 hours under ether anesthesia, at regular intervals. The pharmacokinetic data was evaluated and collected using the PK solver software add-in of Microsoft Excel.

Sample Preparation[24]

The blood/ plasma was immediately centrifuged at 2800 rpm and 4°C temp. (the Remi centrifuge, made in India) for a period of 15 minutes. The separated plasma was kept at temp. -20°C for subsequent HPLC examination done. From the plasma, the drug was removed using the liquid-liquid extraction method. Heparinized rat plasma/blood was centrifuged using an Eltek India RC4100F cooling centrifuge at 2800 rpm for 15 minutes at 4°C,

After the plasma/blood of the rat was removed, an equivalent vol. of HPLC-grade acetonitrile (ACN) was added. After the proteins were softly precipitated, the drug samples were vortexed for at least fifteen15 minutes to properly mix the plasma with the solvent. Further, dilute the supernatant, dichloromethane (DCM) was added, with the amount of dichloromethane DCM being equal to the combined amounts of plasma of sample, and ACN 20 µL was injected into the injector port of the HPLC, prepare or setup already according to a previously developed procedure for the drug analysis/ quantification.[25] The peak region of the graph was used to extract the aloin drug percentage present in the solution sample using HPLC software named Breeze 2. Elevated numerous recorded drug concentrations in plasma previously entrenched the bioanalytical curve of the calibration. The pharmacokinetic (PK) characteristics were established with the aid of the Microsoft Excel add-in PK Solver version 2.0. The computation of the variables using non-compartmental intravascular analysis

Table 1: List of both dependent and independent variables in central composite design

Independent factors variables	*Levels used real value and coded value*		
	Low value (-1)	*Intermediate value (0)*	*High value (+1)*
X_1 Drug (mg)	40	60	80
X_2 Polymer (mg)	50	100	150
X_3 Surfactant (%)	1	2	3
X_4 Stirring time (min)	10	100	150
Dependent variables	Constraints		
Y_1 Particle size (nm)	Size NM (80-215)		
Y_2 Entrapment efficiency (%)	EE % (60-100)		

HPLC Analysis of the Drug

HPLC (Waters, USA) measured the quantity of aloin. Waters1525 pump, manual injector, C18 -column, and detector (photodiode) were the components of the HPLC system, and all components were analytically validated.

ACN as acetonitrile (80:20) is the Milli Q water for the mobile phase.

1-mL per minute: Run rate of HPLC.

35.1°C: Column temperature was kept.

254 nm: Detection wavelength.

Using Breeze2 software integrated the peak area of the drug and its retention time (RT) and also measured the percentage of drug content at overall experiments. Aloin std. calibration curves (SCC) were drawn from 1000 to 5000 ng mL^{-1} of aloin for *in-vitro* samples and from 1000 tom 8000 ng mL^{-1} for *in-vivo* drug aloin samples in the required medium. Phosphate buffer solution (PBS) 7.4 medium was used to prepare *in-vitro* samples produced in spiked plasma *in-vivo* experiments. The data was taken down and calculated. Drug retention time and drug detection were established. The equation $y = mx + c$, with r as the correlation coefficient, y representing peak area ratio, and x being the drug concentration in ng/mL, was used to derive the mean calibration curve.[26] The result was collected and calculated using a software analyzer on the Shimadzu controlled. Linear regression was utilized to find the data with the greatest fit for the calibration curves.

Design, asses, and evaluation of HPLC analytical method of drug aloin

HPLC (Waters, USA) was used to measure the quantity of aloin. Waters pump (Waters, USA), manual injector, column C18, and detector were all components of the HPLC system. Acetonitrile (80:20) is the Milli Q water for the mobile phase. Run time of sample 1-mL per minute.

Column temperature (temp.) was maintained at 35.1°C.

254 nm is the detection wavelength.

HPLC peak area of the sample and RT were combined using Breeze2 software, and the drug content was always determined using this method.[27] Standard calibration curves in the required medium were plotted for both *in-vitro* drug aloin samples and drug *in-vivo* samples, from 1000 to 5000 ng mL^{-1} of aloin and 1000 to 8000 ng mL^{-1}, respectively. Samples for *in-vitro* testing were created in PBS 7.4 medium, whereas those for *in-vivo* testing were created in spiked plasma.[28]

- *Range and linearity*

Seven-point calibration curves for aloin in the conc range of 1000 to 5000 ng/mL were built, and regression parameters, including slope, correlation coefficient, and intercept of this plot were established.

- *Precision and accuracy*

It was estimated by performing duplicate analyses of standard samples at three distinct concentrations that fell within the calibration range. The method's accuracy (% recovery) was discovered to be 100 ± 5%, showing a reasonable agreement between the real and obtained numbers. The RSD described relative standard deviation values for the inter- and intraday precision values were obtained/found to be <3%, showing the new method's repeatability and intermediate accuracy.

Specificity and sensitivity

Proposed technique should be sensitive enough to detect the aloin-drug lower concentration in the samples, and the drug's peaks should be distinct and unaffected by other peaks when used for drug sample analysis. The method's specificity was assessed by injecting dose forms. The conflicting other compound peaks at the corresponding RTs of the sample show that the established approach was sufficiently specialized. The method relies on 2 responses one is the curve of the slope, also termed the standard curve (ST), and another relies on the response's standard deviation (SD). The limit of detection (LoD) and limit of quantification (LoQ) parameters evaluated the sensitivity.

LoD = 3.3 SD / Slope

LoQ = 10 SD / Slope

Data analysis

Pharmacokinetic experiments were conducted using a non-compartmental method. The statistical analysis of the level was set at $p < 0.05$. C_{max} denoted the peak plasma concentration of the drug, AUC denoted area under concentration, MRT indicates the mean residence time of aloin, T_{max} was preferred as the time of its occurrence, AUMC, and $t_{1/2}$ were studied as pharmacokinetic parameters using the linear trapezoidal method. USA-made GraphPad Prism software was used to collect the data statistically. The aloin formulation of collecting data of p. kinetics, including the above parameters and another parameter is half-life $t_{1/2}$, Kel elimination rate of aloin drug, $AUC0_{-t}$, and $AUC0_{-\infty}$, the area under the curve of aloin zero to infinity is presented and measured in below table as a mean, measure of dispersion (SD), Statistical analysis was defined as a *p-value*.

RESULTS OF DRUG ALOIN

The optimum formulation of the aloin PLGA nanoparticle is produced using the nanoprecipitation process. The optimised formulation had a nanoparticle size of 98 ± 0.517 nm, and entrapment efficiency (EE) of about 98 ± 0.698% (Show in Graphs 1-3). Table 2 of 21 experiments tests was conducted by taking 5-center values due to the accuracy and error chance being less. Results show the 4-factor value with - 2-optimized responses. The optimized response of drug aloin tests was characterized by the help of stat-ease software that would evaluate the data statically.

The optimized formula of aloin and predicted response of drug aloin via the central composite response surface drug design method were closely matched.[29,30] The prepared aloin nanoparticle evaluated parameters of the *in-vitro* test in Table 3 and Figure 1, and the stability parameter discussed in Table 4. The kinetics study was characterized by the use of two important software, as mentioned and discussed in the study.

The nanoparticle demonstrated good sustained drug release up to 24 hours after being prepared. First, the bioavailability

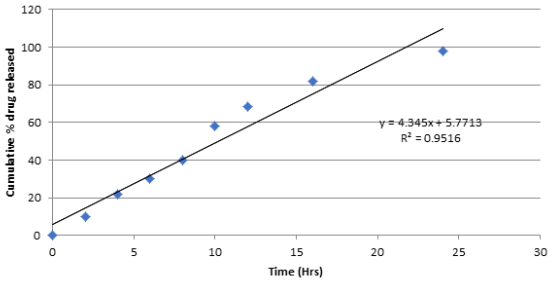


Figure 1: The graph of aloin-loaded PLGA nanoparticle showed the zero-order kinetic

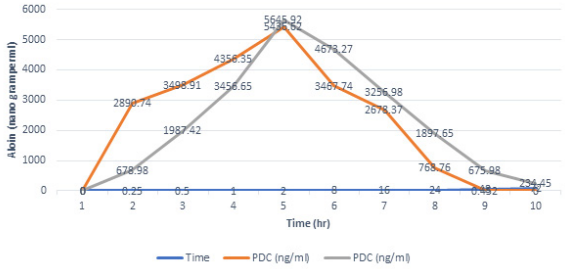


Figure 2: Aloin amount in the plasma of rats following an oral dose of aloin-loaded nanoparticles and suspension of aloin as a standard drug (aloin mg/kg body weight). Nano data were shown

Table 2: Observed response in central composite design for aloin nanoparticles and experimental responses. central composite design represents 21 experiments based on different concentrations of four variables

		Factor one (1)	*Factor two (2)*	*Factor three (3)*	*Factor four 4*	*Response one*	*Response two*
Std	*Run*	*A: drug*	*B: Polymer*	*C: surfactant*	*D: Time*	*Particle size*	*EE%*
		mg	*Mg*	*%*	*Minutes*	*Nanometer*	*%*
21	1	0	0	0	0	99.1	94.7
10	2	1.68	0	0	0	104	87.91
20	3	0	0	0	0	98.5	98.06
1	4	1	1	1	-1	205.3	63.71
4	5	-1	1	-1	1	92.1	89.54
12	6	0	1.68	0	0	148.7	79.52
8	7	-1	-1	-1	-1	161.2	67.7
2	8	1	1	-1	-1	215.3	63.9
7	9	-1	1	1	1	90.1	97.56
11	10	0	-1.68	0	0	129.1	83.1
6	11	-1	-1	1	-1	156.5	86.95
13	12	0	0	-1.68	0	106.8	78.2
9	13	-1.68	0	0	0	113.3	77.73
16	14	0	0	0	1.68	143.6	84.16
15	15	0	0	0	-1.68	195	81.23
18	16	0	0	0	0	108.1	94.72
5	17	1	-1	-1	1	86.5	70.67
3	18	1	-1	1	1	81.3	91.08
17	19	0	0	0	0	98.5	98.06
14	20	0	0	1.68	0	111.2	93.19
19	21	0	0	0	0	98.1	91.58

of nanoparticles and normal drug suspension were contrasted. The average plasma aloin concentrations following oral administration of one dose of aloin suspension to rats. The pertinent pharmacokinetic data are covered in including C_{max}, elimination constant (Ke), T_{max}, ($t_{1/2}$) half-time of elimination, mean residence time, and (Fr) represent relative bioavailability, which is provided in Tables 5, 6 and 7. The concentration-time details of the drug preparations were best suited to a one-compartment model with a weight of 1, and these results are shown in Figure 2.

As per the below in results, the C_{max} value of aloin nanoparticles was bigger than that of suspensions (5645.92 63.3 *vs.* 5436.62 3.3 ng/mL). When the AUC of the nanoformulation (131436.285 ng/mL h) exceeded that of the suspension (82338.5450 ng/mL h), it rose by roughly 20%. PLGA has

Table 3: *In-vitro* study of nanoformulation

Formulation	*Models*	*Kinetic parameter values*
	Zero (0)	$R^2 = 0.951$
	First (I^{st})	$R^2 = 0.936$
	Higuchi	$R^2 = 0.932$
	Korsmeyer Peppas	$R^2 = 0.908$

Table 4: Stability study of nanoparticle

S. No	*Time (Days)*	*25 ± 2°C and 60 ± 5% RH*			*40 ± 2°C and 70 ± 5% RH*		
		Particle size	*EE (%)*	*Zeta potential (mv)*	*Particle size*	*EE (%)*	*Zeta potential (mv)*
1.	0	98.5 ± 1.4	98.06 ± 1.0	-9.5	98.5 ± 1.4	98.06 ± 1.4	-9.5
2.	30	98.5 ± 1.0	98.01 ± 1.2	-9.3	98.4 ± 1.3	98.02 ± 1.0	-9.
3.	60	99.1 ± 1.5	98.08 ± 1.4	-9.7	98.6 ± 1.5	97.99 ± 1.2	-9.2
4.	90	98.9 ± 1.6	98.00 ± 1.4	-9.4	98.9 ± 1.4	97.98 ± 1.4	-9.6

Table 5: Comparison of pharmacokinetic parameters of pure drug and prepared nanoformulation of drug

Parameter	*Aloin*	*Nanoformulation of Aloin*
T_{max} (h)	2	2
C_{max} (ng/mL)	5436.62	5645.92
AUC (ng/mL*h)	82338.54502	131436.285
MRT(h)	10.62360831	21.50392254
AUMC (ng/mL*h^2)	874732.4511	2942120.986
$T_{½}$ (h)	2.472431381	15.91057333
Cl (mg)/(ng/mL)/h	0.00012145	7.30899E-05

Table 6: List of HPLC validation parameters for measuring the pure drug Aloin in PBS 7.4

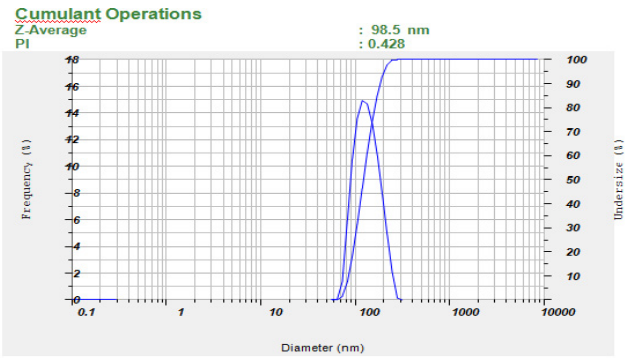
S. No.	*Parameters*	*Aloin*
I.	Wavelength of drug	254 nm
II.	Retention time	7.2 ± 0.76 minutes
III	Slope	14.89
IV	LoD (ng/mL)	380
V	LoQ (ng/mL)	526
VI	Range	1000–5000 ng/mL

been effectively employed in the creation of nanoparticles to increase its bioavailability.[31]

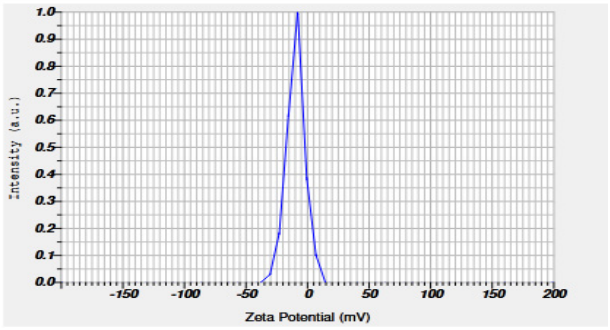
DISCUSSION

In the above research work, an effort has been made to formulate an aloin nanoparticulate formulation. And demonstrates the efficacy of the aloin-loaded nanoparticles produced by the nanoprecipitation technique. This approach relies on increasing shear stress during preparation to diminish the metrics or size of the nanoparticles. This is accomplished by increasing the period of application energy, diminishing the conc. of polymer in organic solvents and surfactant addition in adequate amounts, therefore increasing the volume of the organic phase to the ratio of the aqueous phase. To improve aloin's oral dissolution rate, PLGA is a promising polymeric component for use in the development of innovative drug aloin delivery systems.

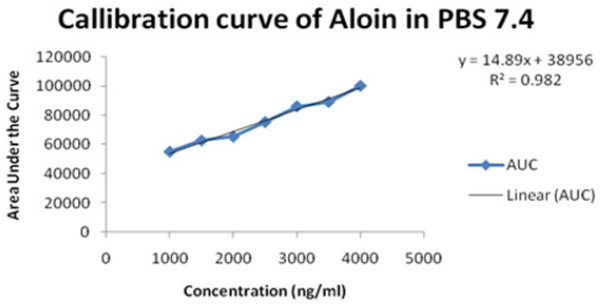
The zeta potential, drug release, and average particle size of each nanoformulation (F1-F21) were all assessed. According to zeta potential analysis, all formulations were stable, and the range of average particle sizes was between 80 and 216. In a 7.4 pH phosphate buffer, *in-vitro* drug release was investigated; formulations employ controlled release to slow metabolism and increase bioavailability.[32] After data treatment for dissolution,



Graph 1: Particle size of nanoformulation of aloin



Graph 2: Zeta potential of optimized aloin formulation



Graph 3: Calibration curve of drug aloin AUC *vs* concentration

release behavior observes the zero-order kinetics. The selected optimized formulation was conducted for stability tests that were kept at temperatures of 25° 2°C and 60% RH and 400° 2°C and 70.5% RH for 90 days (3 months) and were analyzed for their assay, electro kinetic potential (ζ), particle size, and entrapment efficiency, among other things. Throughout

Table 7: Inter and intraday accuracy and precision for analytical method pharmacokinetic validation parameter by HPLC

Concentration (ng/ml)	*Accuracy*		*Precision*	
	Interday	*Intraday*	*Intraday*	*Interday*
1500	100.43 ± 1.176	102.44 ± 1.43	1.89	2.11
2000	103.22 ± 2.01	101.87 ± 1.52	1.67	1.81
2500	102.87 ± 1.98	100.56 ± 1.12	1.62	2.01

the stability period, there was no discernible change in the physicochemical parameters of the formulation of the nanoparticles. The bioavailability research of the aloin drug was also assessed successfully.

CONCLUSION

With this, the aloin-PLGA NP was optimized using the synthesized approach and had an EE of 98.09% and a size of 98 nm, showing strong drug delivery ability. An extended drug release profile and value-added pharmacokinetic research are also included. It was determined that aloin-loaded PLGA nanoparticle formulation was successfully studied with the four-factor, two-level, five-center in central composite surface design drug stat design using the 13.01 trial version optimized the results and produced the best process conditions. The research data that the PLGA nanoparticulate drug delivery (DD) system loaded with aloin using the nano pptation. method by selecting independent and dependent variables was an optimized and reproducible method. The formulation of aloin nanoparticulate exhibits an improved dissolution rate by increasing the AUC of the NP aloin-drug in the plasma profile, according to the *in-vivo* pharmacokinetic data. More research involving several animal species is advised to look into the therapeutic effect of this formulation.

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