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EFFECT OF NANOPATICLES ON PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF ANTIMALARIAL AGENTS

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ABSTRACT

Nanoparticles play an important role in a wide variety of fields including advanced materials, pharmaceuticals, and environmental detection and monitoring. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Many therapeutic agents have not been successful because of their limited ability to reach to the target site. There are various aspects of nanoparticle formulation, characterization, effect of their characteristics and their applications in delivery of drug molecules and can be exploited to achieve maximum potential of therapeutic agent . In present study designing nanoparticles , as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen against *Plasmodium* in mice model. Extracts of *Azadirachta indica, Cinchona officinalis* and *Artemisia annua* having anti plasmodium activities and chloroquine, mefloquine, artesunate synthetic drugs were selected in the study. Results obtained signifies the ability of nanoparticle to entrap hydrophilic and hydrophobic drugs with concomitant reduction in their toxicity potential, their versatility and their amenability for surface modification are the major factors responsible for their popularity in drug delivery research.

KEYWORDS: Nanoparticle , pharmaceuticals, pharmacokinetic , pharmacodynamic, antimalarial

1. INTRODUCTION

Nanoparticles are subnanosized colloidal structures composed of synthetic or semi synthetic polymers. Depending on the method of preparation, nanoparticles, nanopspheres or nanocapsules can be obtained with different properties and release characteristics for the encapsulated therapeutic agent.Nanocapsules are vesicular systems in which the drug is confined to acavity surrounded by a unique polymer membrane, whereas nanopspheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol), have been used as potential drug delivery devices because of their ability to circulate for a prolonged period of time.(Vyas *et al.*, 2002).

2. MATERIAL AND METHODS

Plant material

Plant selected for antiplasmodium activity: *Artemisia annua, Cinchona officinalis, Azadirachta indica* . All the plants were authenticated and herbarium was deposited at department of pharmacy, Barkatulah University,Bhopal.

Preparation And Extraction Procedure For Plant Material

Prepration of plant material.

Materials of the three plants selected for the present study were grinded to coarse powder and stored in airtight containers at room temperature in the dark until used.(Trease and Evans 1978).

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Method For Alcoholic Extraction

Extraction for Cinchona officinalis

The powdered *Cinchona* bark (50g) was mixed with calcium oxide (30gms), water (40 ml) and 5% sodium hydroxide to form a paste and kept overnight.Then obtained paste was packed in Soxhlet apparatus and extracted with methanol for 8 hrs at 60ºC and processed as adopted by Kokate, 2009, detailed method was given in Deshpande *et al* 2014 .Then stored at 4ºCuntil use in sealed bottle, protect against light and moisture.

Extraction of Azadirachta Indica

50 g of air-dried powder was soaked in ethyl alcohol (100 ml) and kept for over night.Then it was filtered through 8 layers of muslin cloth, filtrate was collected and residue was discarded. Filtrate was packed in soxhlet apparatus and extraction was done for 8 hrs at 80-90ºC.Solvent was distilled off to one –fourth to yield alcoholic extract(Deshpande *et al* 2014).

Extraction for Artimisia annua

Artemisinin, a sesquiterpene lactone is an antimalarial constituent of *Artimisia annua*. Extraction process which was followed in present study is: Dried leaves of artimisia annua are coarsely powdered and extracted with the help of petroleum ether (over night). Petroleum ether was then filtered then concentrated to dryness. Concentrated extract found was then dissolved in chloroform. Aerial part of *Artemisia annua* were dried in shade, grounded to coursed powder stored in air tight amber colored bottle. Artemisinin, a sesquiterpene lactone is an antimalarial constituent of *Artimisia annua*. Extraction process which was followed in this study. Dried leaves of *Artimisia annua* were coarsely powdered and extracted with the help of petroleum ether (over night). Petroleum ether was then filtered then concentrated to dryness with the help soxhlet then concentrated extract found was then dissolved in chloroform. Acetonitrile was added to the chloroformic solution which is responsible for the separation of impurities and pricipetation of waxes. Filtration was done to separate out the impurities. The filtrate was again concentrated and was subjected to cool down. Crystals of artimisinin were deposited which were further purified by washing and recrystallization with alcohol.(Trease and Evans 1978, Kokate, 2009 , Deshpande *et al* 2014).

Method for Aqueous extraction

The powdered material of all the selected plants (50g) separately were soaked in water (100 ml) and kept for overnight. Then it was filtered through 8 layers of muslin cloth, filtrate was collected and residue was discarded. Filtrate was packed in soxhlet apparatus and extraction was done for 6- 8 hrs at 80-90ºC. Solvent was distilled off to one –fourth to yield aqueous extract. Collected extract was then weighed and stored at 4ºC until use.

Preparation and optimization of chitosan nanoparticles

Chitosen nanoparticles were prepared with suitable modification based on the ionotropic gelation with Tpp anions. Chitosan (2mg ml^{-1}) was dissolved in aqueous acetic acid (pH 4.0) solution and Tpp was dissolved in distilled water at the concentration of 1mg/ml. Drug (10%) was added to Tpp solution (by dissolving Tpp in 100ml distilled water under continuous stirring on magnetic stirring).Finally 1.5 ml of drug containing Tpp solution was added to 4 ml of chitosan solution with the help of syringe needle under magnetic stirring at room temperature.The dispersion so formed was sonicated for 15 minutes, then disperse in water and centrifuge the sample at 12000 rpm for 30 minute. The supernatant was discarded and pallet was resupended in distilled water and lyophilized and stored at -20ºC till further use, and sample was further characterized.

Study of process variables and optimization involve in chitosan nanoparticles.

The number of variable observed in preparation of nanoparticles.They have different properties like surface morphology, particle size, drug content and release profile. For optimization of nanoparticle different parameter which are under consideration in this research work.

- Polymer concentration.
- Effect of acetic acid concentration.
- Speed of magnetic stirrer.

Effect of TPP concentration

Drug encapsulation efficiency.

The entrapment efficiency of drug and extracts was determined by using following equation.

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$$\textbf{\textit{Encapsulation efficiency}}\ (\%) = \frac{\textbf{\textit{Total drug}} - \textbf{\textit{Free drug}}}{\textbf{\textit{Total drug}}} \times \mathbf{100}$$

About 10ml of nanoparticulate suspension was digested with minimum amount of ethanolic solution (Water: ethanol in 7:3 ratios). The digested homogenates were centrifuged at 12000 rpm for 30 min and supernatant was analysed for drug entrapment. Entrapped drug and extracts in this study were measured at their respective λ maximum.

***In vitro* release studies**

In vitro release of hydrophilic molecule out of nanoparticles was measured spectrophotometrically by incubation of liposomes in 1OmM Tris buffer (pH: 7.4) at 37°C in mild shaking (35 rpm) water bath under mild-shaking conditions for determined time intervals. Dialysis bags (Dialysis membrane 110, Hi Media, India) were immersed in water for one hour to remove the preservatives followed by rinsing in phosphate buffered saline (PBS) solution. The drug and extract encapsulated nanoparticles were placed in PBS and loaded in the dialysis bag. The bag was sealed at both the ends and immersed in 4 mL of PBS with 10% methanol (Katrin, 1995). The release of the drug and extract was evaluated at three different pH values (1.2, 7.4 and 9.0). A pH of 1.2 was maintained using 0.1 M HCl -KCl buffer while pH 9.0 was maintained using 0.1 M phosphate buffer. Samples were withdrawn at time intervals of 0.5, 1, 2, 4, 6, 8, 24-h, periodically(Cabral *et al* , 2004)

Morphological characterization

The morphology of the nanoparticles was determined using a scanning electron microscope (JEOL 6701F, from AIIMS New Delhi).The samples were placed over a carbon paste coated stub and sputter coated with a thin layer of platinum prior to viewing. For negative staining, 2% (w/v) phosphotungstic acid was added to the liposome samples and incubated at room temperature for 24 hours. This sample was freeze dried and imaged using scanning electron microscope. The transmission electron micrographs of the liposomes were obtained using JEM 1011, JEOL. The lyophilized liposome sample was dispersed in 0.5 mL PBS. To 50 µL of this dispersion, an equal volume of double distilled water was added and placed on a carbon coated grid. The excess water was absorbed using a filter paper and uranyl acetate stain was added. The grid was then washed with water to remove excess uranyl acetate and then dried before it was loaded in the specimen chamber.

3. RESULTS AND DISCUSSION

All extracts which were to be screened (Deshpande *et al* 2015)for the anti plasmodium activity *in vitro* model were resuspended in DMSO (1mg ml-1 stock), dilutions was prepared from the stock (under aseptic conditions) all doses were chosen based on a pre dose finding test for extracts from the literature values for plant. LD_{50} obtained for each extract was calculated.Similarly synthetic drugs were screened for activity against *P.falciparum* culture maintained in laboratory. Culture was synchronized with sorbitol treatment before starting the screening protocol. LD_{50} of drugs are calculated with probit graph .

Optimization of chitosan nanoparticles

Polymer concentration

Chitosen solution of different concentration (20mg $10ml^{-1}$ - 40mg $20ml^{-1}$) was prepared to study the effect of polymer concentration on particle size.

Effect of acetic acid concentration

Acetic acid concentration was varied from 0.5-2% to study the effect on particle size.

Speed of magnetic stirrer

Speed of magnetic stirrer was varied from (1000-5000 rpm) to study the effect on particle size.

Effect of TPP concentration

The cross linking agent concentration was varies from 1mg/ml to 3mg/ml to study the effect on particle size. Different batches were prepared by inotropic gelation method by varying the parameters mentioned above and their effect on different properties was observed and summarized. From the above observations better results were obtained when polymer concentration was 20mg $10ml^{-1}$, Cross linking agent concentration was 1mg/ml,

acetic acid 1% and the speed of magnetic stirrer 4500 rpm. Results of variables and their effect (Table 1) on nanoparticle size is discussed after morphological description of nanoparticles.

Surface morphology

TEM was carried out to investigate the morphology of nanoparticles.TEM images reveals that drug and extract loaded nanoparticles displayed a spherical shape with smooth surface and no aggregation were observed. No differences in morphological properties were reported between drug/extract loaded nanoparticles and empty nanoparticles. Nanoparticles with narrow size distribution could be achieved by appropriate stirring strength in preparation process (Fig. 1a ,1b) TEM images of nanoparticles obtained.

Percent encapsulation efficiency

Nanoparticulate suspension was digested with minimum amount of ethanolic solution. Then digested homogenates were centrifuged at 12000 rpm for 30 min and supernatant was (Table: 2) analysed for drug entrapment. PBS of pH 7.4 was used as suspension media. Entrapped drug and extracts in this study were measured at their respective λ maximum.

λ max of *Azadirachta indica* extract : 577nm,for *Cinchona officinalis* extract : 250nm, for *Artemisia annua* extract: 365nm,for Chloroquine: 343 nm, for Mefloquine:283nm ,for Artesunate : 287 nm.

Cumulative percentage of *in vitro* release of extract encapsulated in nanoparticle

Extract encapsulated in nanoparticulate was analyzed for *in vitro* release pattern. The extract encapsulated nanoparticles were placed in PBS and loaded in the dialysis bag. The bag was sealed at both the ends and immersed in 4 ml of PBS with 10% methanol (Katrin, 1995). For each extract release test was repeated for three times and cumulative release (Table 3 and Fig: 2) value for each extract was determined. For analysis reading were recorded at λ max of each drug and extract. Entrapped drug and extracts in this study were measured at their respective λ maximum for *Azadirachta indica* extract : 577nm,for *Cinchona officinalis* extract : 250nm, for *Artemisia annua* extract: 365nm,for Chloroquine: 343 nm, for Mefloquine:283nm ,for Artesunate : 287 nm. Cumulative release value for each extract is given in Table 3.

Cumulative percentage of *in vitro* release of synthetic drugs encapsulated in nanoparticles

Synthetic drugs encapsulated in nanoparticulate were analyzed for *in vitro* release pattern. The drug encapsulated nanoparticles were placed in PBS and loaded in the dialysis bag. The bag was sealed at both the ends and immersed in 4 ml of PBS with 10% methanol (Katrin, 1995). For each drug release test was repeated for three times and cumulative drug release (Table 3) value for each set was determined .Cumulative release value for each drug is given below. Graphic representation for cumulative drug release of nanoparticle entrapped drug and extract is given in Fig. 2.

4. CONCLUSION

In present study we had obtained nanoparticles of 100 to1753 nm in size, with efficiency upto 90% with plant extracts and synthetic drugs, showing smooth surface when studied by TEM, no morphological defects were observed in TEM study. *In vitro* release pattern is uniform and controlled, and follows first order of kinetics. Similar results were reported by various authors in their study related to nanoparticle as a tool for drug delivery.

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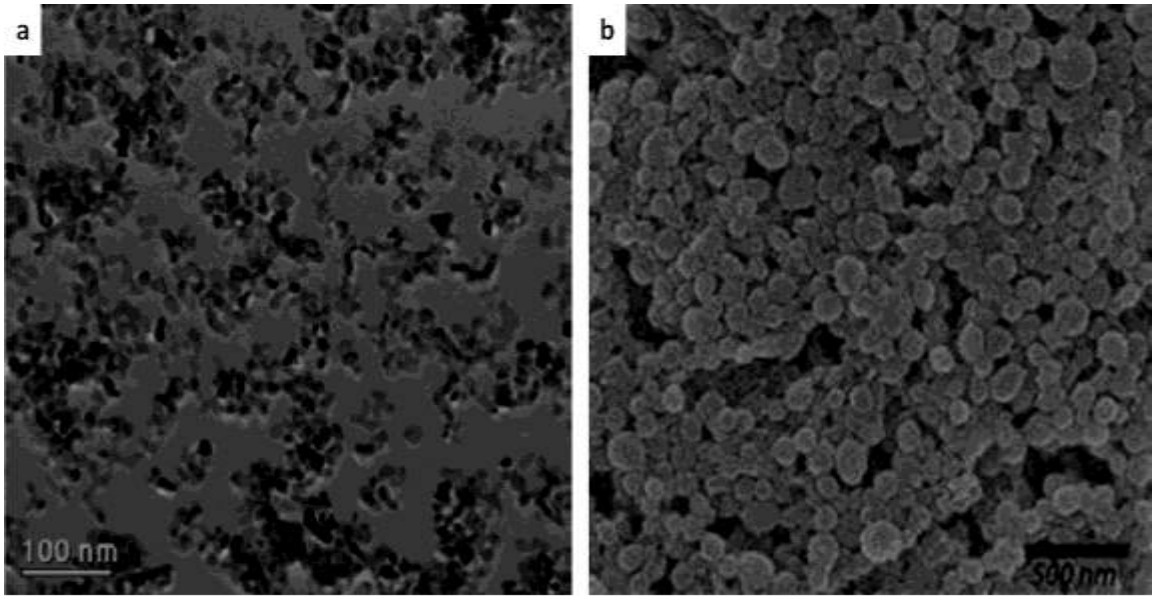

Fig. 1: TEM images of nanoparticles empty (a), with drug (b), (magnification 60000x).

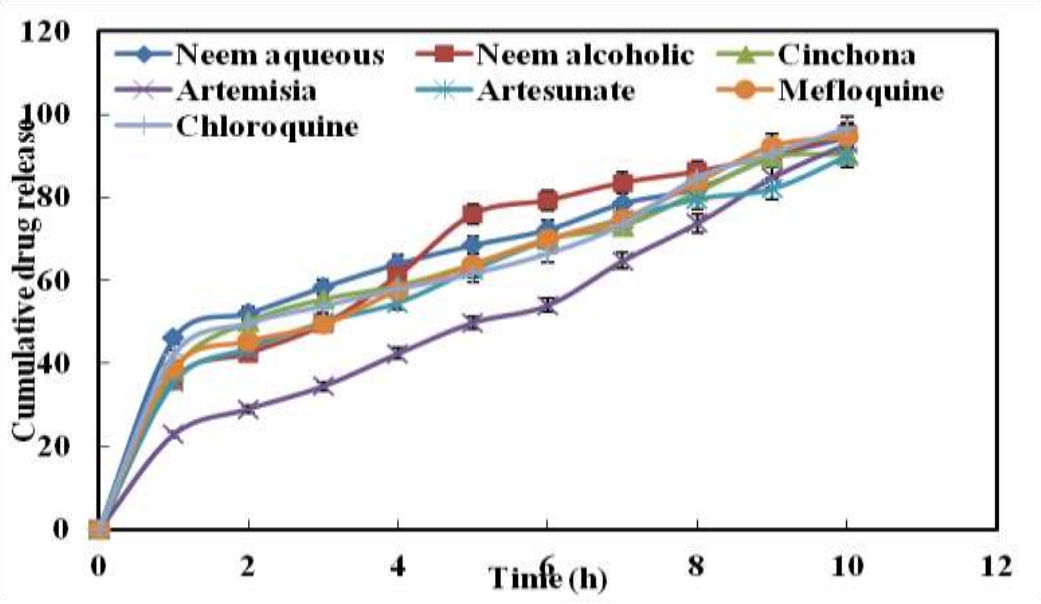

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Fig.2: Cumulative drug release from nanoparticles in vitro model

Table 1: Effect of variables on the particle size

S.No	Polymer concentration	Acetic acid concentration	Speed of Magnetic stirrer	TPP concentration	Particle size Nm
1	20	0.5%	2000	1	1654
2	30	0.5%	2000	1	1662
3	40	0.5%	2000	1	1753
4	20	1%	4500	2	100
5	30	1%	4500	2	154.32
6	40	1%	4500	2	223.65
7	20	2%	1000	3	296.54
8	30	2%	1000	3	N F
9	40	2%	1000	3	NF

Table 2: Results for cumulative in vitro release of extract in present study encapsulated in nano particles

S.No	Time in hrs	Cumulative %release *Azadirachta indica* aqueous extract	Cumulative %release *Azadirachta indica* Alcoholic extract	Cumulative %release *Cinchona officinalis* extract	Cumulative %release *Artemisia annua* extract
1	1	46.07	35.85	38.26	22.89
2	2	52.08	42.45	49.98	29.06
3	3	58.43	49.35	55.43	34.54
4	4	64.12	61.24	58.65	42.43
5	5	68.56	75.98	63.87	49.87
6	6	72.32	79.31	69.98	54.05
7	7	78.65	83.54	73.10	64.76
8	8	82.30	86.23	81.25	73.86
9	9	89.85	90.12	89.56	84.87
10	10	94.54	95.23	90.32	93.05

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Table 3: Cumulative percentage of in vitro release in present study of synthetic drugs encapsulated in nanoparticles.

S.No	Time (h)	Cumulative drug %release Artesunate	Cumulative drug %release Mafloquine	Cumulative drug %release Chloroquine
1	1	35.45	38.93	42.04
2	2	43.67	45.32	49.63
3	3	49.97	49.42	53.75
4	4	54.65	57.64	57.85
5	5	62.65	63.65	61.53
6	6	69.76	69.95	66.38
7	7	74,84	74.84	73.34
8	8	79.64	83.73	84.72
9	9	82.08	92.43	90.43
10	10	89.95	94.73	96.63

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