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Research Article

Design and *in vitro* characterization of Amoxicillin loaded sepia nanoparticles

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ABSTRACT

The objective of the present work was to investigate the preparation of sepia nanoparticles as a potential drug carrier, amoxicillin Trihydrate as a model drug. The sepia nanoparticles were prepared by using the controlled gellification process. In this research work, different drug and polymer ratios were used. The prepared nanoparticles were evaluated to assess the various parameters such as drug content analysis, particle size analysis (SEM Analysis), Zeta potential analysis, *In-Vitro* drug release and stability studies. The particle size of prepared nanoparticles was found to be 500nm. The drug content analysis of all the prepared formulations was estimated in the range of 57.34 ± 0.10 to 65.33 ± 0.11 %. The zeta potential of the sepia nanoparticles was estimated as 69mv. The Cumulative percentage drug release of the third formulation was found to be 90.36 %. By observing *in- Vitro* drug release results of all formulations, we concluded that, AMN3 formulation was found to be the best formulation with a higher Cumulative percentage drug release.

Keywords: Amoxycillin, Sepia Nanoparticles, Zeta Potential Analysis, SEM Analysis, Particle Size, U.V. Spectroscopic analysis.

INTRODUCTION

Nanoparticles are defined as particles of less than 1000 nm in diameter. Sepia melanins are negatively charged pigments that are hydrophobic, containing phenolic or indolic compounds. These are dark in colour and they are used in the preparation of U.V-absorbing optical lenses, cosmetic creams, pharmaceutical formulations, in the preparation of pastries and drug delivery systems in nanotechnology.

Amoxycillin is a broad spectrum antibiotic effective against various types of microorganisms, but it possesses a short biological half life i.e. 60 minutes. Hence, repeated administration is needed to maintain the blood plasma concentration of amoxycillin (Madamwar. et al., 2007). In order to reduce the adverse effects due to frequent dosing, there is a need of a controlled release formulation. In this present study, the controlled release formulations of Amoxycillin were prepared and the *in-Vitro* evaluation was done. The nanoparticles of amoxycillin was prepared by using naturally available, nontoxic, cheap polymer obtained from the marine mollusc i.e. Sepia officinalis which, contain an ink gland in the abdomen and secrete a viscous ink like fluid. The ink was collected and dried and the powder form of this ink was used for the preparation of nanoparticles.

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MATERIALS AND METHODS

Amoxycillin Trihydrate was obtained from Tini Pharma, Tirupati, as a gift sample. Chitosan was obtained from Fisheries College and Research Institute, Toothukudi, Tamilnadu as a gift sample. Sepia (polymer) was procured from local fish vendors of Toothukudi and authenticated by Dr. R. Santhanam, Retired Professor, Fisheries College Thoothukudi, India. All chemicals used were of analytical grade.

PREPARATION OF NANOPARTICLES

The sepia nanoparticles were prepared by the controlled gellification process (Kuller, et.al, 2006) which is already in application for the encapsulation of antibiotics. Initially, the sepia powder was taken, and it was mixed with the drug amoxicillin solution. To this, 1ml of calcium chloride (3 %) solution was added and mixed thoroughly by stirring with the magnetic stirrer for about 15minutes. Then 1ml of chitosan solution (1 %) was added and the stirring was continued for another one hour. Finally, the resultant solution was sonicated in the organ sonicator for 30 minutes. This mixture was kept for one day at 25 - 30°C. The drug loaded nanoparticles were harvested by centrifugation at 16000

rpm by Remi centrifuge for one hour. The flakes were formed, collected, dried and stored in a suitable container. The initial concentration of the drug polymer ratio was 1:1 w/w. Likewise different concentration of drug and polymer were prepared. The composition of the nanoparticles formula was mentioned in the Table 1.

Table 1: Composition of the formulation for sepia nanoparticles containing amoxicillin

S.No	Batch Code	Drug Polymer ratios (D : Sepia Polymer)	Calcium Chloride Solution (3%)	Chitosan solution (1%)
1	AMN1	1 : 1.0	1 ml	1 ml
2	AMN2	1 : 1.5	1 ml	1 ml
3	AMN3	1 : 2.0	1 ml	1 ml
4	AMN4	1 : 2.5	1 ml	1 ml
5	AMN5	1 : 3.0	1 ml	1 ml
6	AMN6	1 : 3.5	1 ml	1 ml
7	AMN7	1 : 4.0	1 ml	1 ml

PARTICLE SIZE ANALYSIS

Particle size distribution was determined by using scanning electron microscope (SEM). A suspension of nanoparticles was sprayed on to a glass cover slip with the aid of an atomizer. (Fuying cui, et al., 2006). The fine droplets were dried over night. Spultened with about 100 mm thick gold layer and then size was determined by quasi-electric light scattering technique (M. Garsia – Fuentes, et al., 2002).

ZETA POTENTIAL ANALYSIS

The zeta potential was measured on dispersions of nanoparticles batch, diluted with an aqueous solution NaCl (0.9% W/V) using zetasizer (Giacomo fontane, et al., 2001; Cafaggi, et.al, 2007). The zeta potential was determined for the best formulation only. Each sample was analyzed in triplicate.

DRUG CONTENT ANALYSIS

Drug content was analyzed by adding 100ml of 10 % HCl with 30 mg of nanoparticles kept at room temperature of about 35°C for 24 hours (Joseph Nisha Mary, et.al, 2006). After that the nanoparticles were separated by centrifugation at 16000 rpm and the drug concentration was analyzed in supernatant liquid by UV-Visible spectrophotometer at 285 nm. The drug content results profile of the all formulations were mentioned in the Table 2.

$$\text{Drug loading capacity} = \frac{Amt.of\ drug\ bound\ by\ total\ amt.of\ Nanoparticles}{Amt.of\ drug\ taken} \times 100$$

***IN-VITRO* DRUG RELEASE STUDIES**

30mg of drug loaded nanoparticles were placed in the USP Dissolution test apparatus basket type stirring element. The basket was covered with an egg membrane. 900 ml of phosphate buffer solution (pH 7.4) at 37°C was used as dissolution medium (Nagai *et al*., 1993; Yang *et al.,* 1999). The basket was rotated at a speed of 100 rpm. A 5ml of medium was withdrawn at various time intervals of 15minutes, 30minutes, 1hr, 2hr, 4hr, 8hr and 24hrs with the help of 5ml pipette and replaced by 5ml of phosphate buffer solution (pH 7.4) the drug content was estimated by UV spectrophotometer at 285nm. The *In-Vitro* drug release profiles were mentioned in Table 3.

Table 2: Determination of the drug content and particle size of prepared sepia nanoparticles

S.No	Batch Code	Drug content (%)
1	AMN1	59.25 ± 0.13
2	AMN2	62.34 ± 0.09
3	AMN3	65.33 ± 0.11
4	AMN4	64.13 ± 0.12
5	AMN5	61.34 ± 0.13
6	AMN6	59.24 ± 0.09
7	AMN7	57.34 ± 0.10

* Average of five preparations ± S.D

Stability study for best formulation

The stability study was carried out using the batch AMN3. The stability of the drug loaded nanoparticles was evaluated in terms of its drug content. Nanoparticle formulation was incubated at 4°C, room temperature, 45°C for one month. The amount of drug was detected UV Spectrophotometrically at 285 nm. The

Table 3: In-Vitro drug release studies of prepared Sepia Nanoparticles

S.No	Time	Cumulative % drug Release						
		AMN1	AMN2	AMN3	AMN4	AMN5	AMN6	AMN7
1	15minutes	41.58	42. 8	47.04	42.18	39.72	38.52	32.24
2	30miutes	42.72	44.1	51.44	47.84	43.56	40. 8	36.41
3	1 hour	46.92	45.4	55.76	50.16	50.76	44. 1	38.24
4	2hours	50.88	47.2	68.52	55.68	52.04	46. 4	41.06
5	4hours	54.92	47.5	77.16	61.38	60.52	49.44	45.32
6	8 hours	59.88	48.2	83.52	65.64	62.34	54.71	49.24
7	12hours	63.24	72.3	85.44	71.76	70.44	63. 6	59.96
8	24hours	68.52	72.5	90.36	85.44	81.36	75.44	62.24

stability study results of the best formulation were mentioned in the Table 4.

Table 4: stability studies of prepared best formulation of Sepia Nanoparticles containing Amoxicillin

S. No	Time in week	AMN 3 Formulation		
		4°C	Room temperature	45°C
1	0	100	100	100
2	1	98.56	97.43	94.75
3	2	97.54	95.56	93.54
4	3	93.42	94.32	92.34
5	4	92.65	93.65	88.86

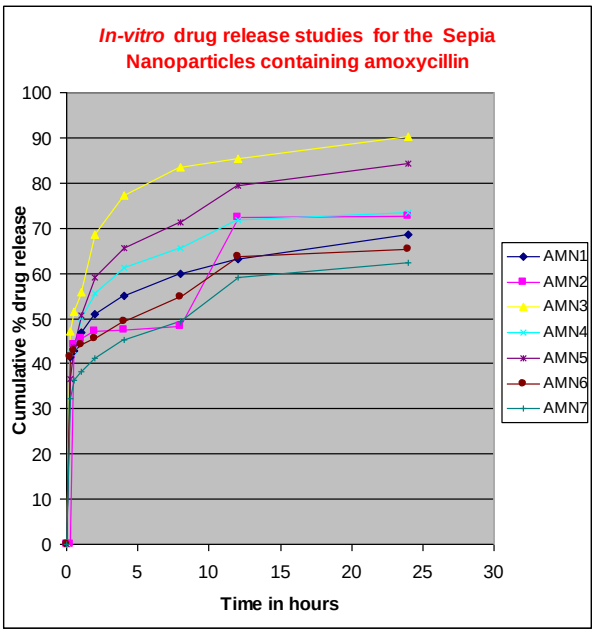

Figure 1: In-Vitro drug release studies for the Sepia Nanoparticles containing Amoxycillin

RESULT AND DISCUSSION

Amoxycillin nanoparticles with varying proportions of Amoxicillin and Sepia were prepared by Gellification method. The particle size of prepared nanoparticles was found to be 500nm. Zeta potential of the best formulation was determined and it was found to be 69mv. The drug content was determined by centrifugation method and it was maximum in formulation AMN3. The nanoparticles exhibited an increase in drug content with an increase in the polymer ratio, up to a particular concentration (1:2). A decrease in drug content was observed after that point due to the saturation capacity of polymer. The stability study was performed for the best formulation, does not showed any remarkable change in the drug content. This indicates the above formulation was stable in storage medium condition. The *In-Vitro* release profile of all formulation is shown in the Table 3 and the *In-Vitro* drug release studies profile for all the formulations are represented graphically in Graph 1. The release of amoxicillin mainly depended upon the polymer concentration. The burst release of amoxicillin from nanoparticles at initial stage resulted from the dissolution of drug crystals on the surface of nanoparticles. As increasing the concentration of the polymer, increase the in-vitro drug release up to certain extent, i.e., polymer proportions (1:2). The cumulative percentage drug release of the third formulation was found to be 90.36 %. By observing the In-*Vitro* drug release results of all formulations, AMN3 formulation was found to be best formulation with higher cumulative percentage drug release.

CONCLUSION

The ratio of drug and polymer concentration 1:2 was found to be ideal. At the 24th hour, the maximum amount of drug was released. As the amoxicillin has a short biological half life, it was used in the controlled released formulation. The prepared nanoparticles releases the drug in a controlled manner and the polymer used was nontoxic, bio compatible and freely available and act as a good carrier of the therapeutic agents.

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