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PREPARATION AND STANDARDIZATION OF LIPOSOMES ENCAPSULATED NEWCASTLE DISEASE VACCINE IN UNILAMELLAR AND MULTILAMELLAR FORMS

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Abstract

Preparation and standardization of a novel Nano-liposomal encapsulated Newcastle disease vaccine by two formulated multilamellar and unilamellar liposome encapsulated vaccine via thin film method. Electron and, light microscope scaled the size and lamellar and entrapment of vaccine were standardized of liposome encapsulated Newcastle disease vaccine. The modified virosome offered qualified entrapment percent in unilamellar 84.95±7.53% and multilamellar 76.75±4.59 %, The modified virosome size of unilamellar encapsulated Newcastle disease vaccine was 150±6.66 and multilamellar encapsulated Newcastle disease vaccine 575±4.04. The focus of this research will be to highlight to preparation and standardization of liposome that are relevant for veterinary medicine protected the vaccine from several passage administration pathways and increase circulatory half-life with increase time of vaccine release.

Key words: vaccine, liposome, Newcastle disease.

Introduction

Recent advances in nanotechnology have allowed for the development of novel nanodrug and vaccine delivery systems such as liposomes. These systems are known to enhance the therapeutic indices of the incorporated drugs or vaccine through a number of ways and protect the entrapped agent from the internal body environment, improve the bioavailability and pharmacokinetics of the drug, are able to evade immune capture allowing for sustained-release over time (Underwood and van Eps, 2012; Irache *et al*. 2011; Onuigbo, 2012).

Among the wide variety of existing drug-delivery systems, several liposome-based therapeutic agents in animals have been evaluated over the past decade and have been demonstrated to be highly versatile and easy to modify and are relatively simple to formulate (Sahoo and Labhasetwar, 2003; Torchilin, 2005). They are spherical self-closed vesicles formed by one or more concentric lipid bilayers around an aqueous inner compartment with therapeutic agents capable of being encapsulated within the aqueous cavity or the lipid bilayers of the liposomes (Torchilin, 2005).

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Vaccination is considered one of the chief developments in modern medicine, as the most effective eradication method ever was developed (OIE, 2015 and Han *et al*., 2018). The Newcastle vaccination is commonly programmed eradication against Newcastle virus and awareness the effective immunization, (Dimitrove *et al*., 2017). Several attempts were done for maximizing vaccination efficiency recent advances in Nano-medicine have been studied in the veterinary field and have found a wide variety of applications (Sadozai and Saeidi, 2013).

One of the major unconventional approaches to enhance immunogenicity is delivering antigens by liposomal encapsulation as derived vehicles. Liposomes have been considered intensively because of their potential as immune-stimulants and their capacity to deliver antigenic components (Ghaffar *et al*., 2014; Gregoriadis, 1990).

The focus of this research will be to highlight to preparation and standardization of liposome that are relevant for veterinary medicine protected the vaccine from several passage administration pathways (Bulbake *et al*., 2017), occupying anew pharmacological modifying compartment threshold of steady-state set point and

increase circulatory half-life (Schmidt *et al.*, 2016 and Chang and Yeh, 2012) with increase time of vaccine release.

Materials and Methods

The experiment was achieved protocol in Collage of Veterinary Medicine and pharmacology Lab. The ethical issued and followed scientific education program (Al-Bayati and Khamas, 2015) of standardizing the laboratory animal in scientific research. The vaccine standardization and immunity were done in the Veterinary Directorate/ Ministry of Agriculture/Iraq.

Vaccines preparation and standardization

The vaccine was prepared and standardized in two different settling methods showed as follows:

• Ordinary vaccines preparation: $ND_{Las.}$ preparation: One vial of lyophilized ND 2500 dose was dissolved in 2 ml PBS 4°C with vortex for 20 sec (Allan, 1978). $ND_{Las.}$ vaccine EID_{50} standardization. The EID_{50} of vaccine was achieved as following settling maneuvers.

• Eggs Inoculation: Fertilized eggs used for propagation of live Newcastle disease virus vaccines strains according to (Trybala, 1987).

• Preparation of red blood cells of the chicken: Blood was collected from the wing vein of chicken and processed according to (Alexander,1998).

• Rapid Slide Agglutination: It was used to detect the growth of the virus in the allantoic fluid (Allan *et al.*, 1978).

• Estimation of EID_{50}: The EID_{50} was measured by titration of vaccine on chicken embryonated egg; 10 days old embryonated chicken eggs were used for titration of the virus. (Rai, 2005). The 10^9 EID_{50}/ 1 ml for the strain was set in vaccination and Nano-liposome preparations.

• Nanoliposomal Vaccine Preparation: The liposomal Nano-sized scale encapsulated vaccine was organized, standardized and achieves bio-enhancer carrier liposome carrying vaccine was done as follows.

• Liposome Preparation Technique: The liposome was formulated according to the Bangham thin film technique (Marie and Habeeb, 2012) of settling steps as follows:

1. Multi lamellar liposome: The mixture of both cholesterol 0.25 g. and 0.25 g. phosphatidylcholine; 1:1w/w were dissolved in a combination of Chloroform 10 ml and Methanol 5 ml 2:1 v/v. fig. 3.2 shows cholesterol and phosphatidylcholine container.

2. Unilamellar liposome: The mixture of both Cholesterol 0.25 g. and 0.25 g. Phosphatidylcholine, 1:1 w/w. The mixture was dissolved in 2 ml methanol prior one drop (50 μl) of Chloroform.

The phosphatidylcholine and cholesterol organic solvent were vortexes continuously for 30 min with evaporation by rotary evaporator; conducted with vacuum pump at 40°C. The yield was a dry thin film, foam-like appearance deposited on tube wall, The vaccine strain was merged in thin-film liposome and vortexes for 30min. 37°C to prepare liposomal vaccine LipoENDV (Liposome encapsulated Newcastle disease vaccine) entrapment.

• Light Microscopic identity of liposome: Liposome smear was prepared 50 μl of 0.1% suspension a (Siuta *et al.*, 2016). Measurements include 100 liposomes. Morphology and particle size were calculated (Cabarl *et al.*, 2004).

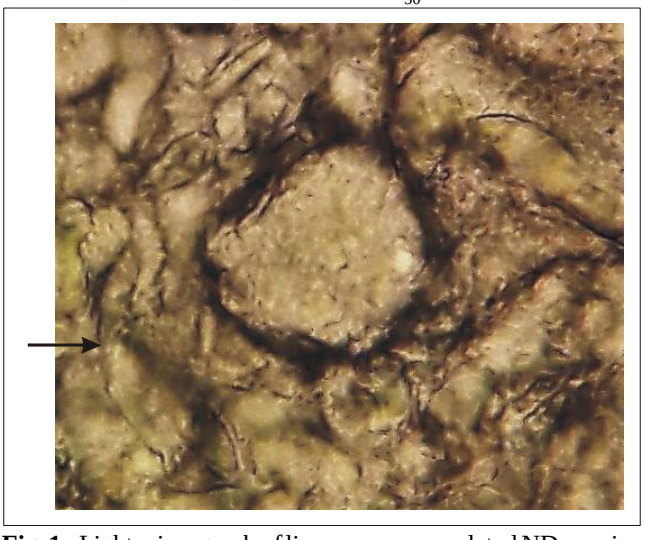

Fig. 1: Light micrograph of liposome encapsulated ND vaccine set by phosphatidylcholine-cholesterol in Methanol: Depict of multi lamellar multi vesicles shape green arrow. X1500.

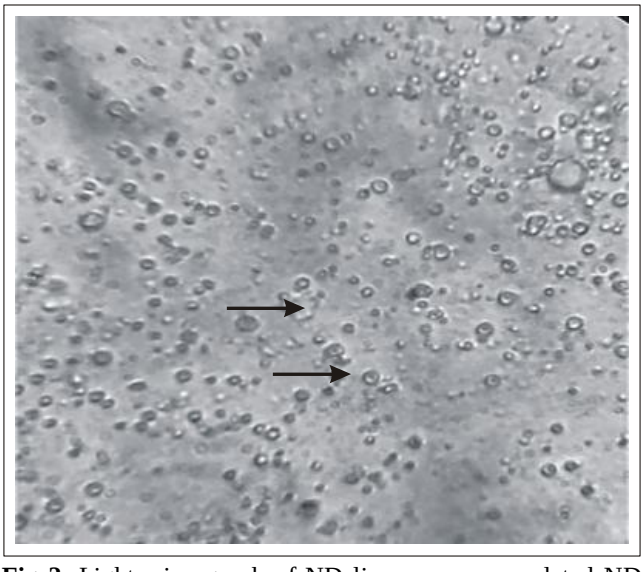

Fig. 2: Light micrograph of ND liposome encapsulated ND vaccine set by phosphatidylcholone-cholesterol in Methanol: Depict of Unilamellar Univesicles shape yellow arrow. X 400.

Table 1: Size, lamellar and entrapment efficiency of encapsulated NDV.

Types of liposome	Size of LENDV nm		Lamellar number of LENDV		Entrapment Efficiency of vaccine
	Mean±SE	Range	Range	Mean±SE	
Multi lamellar liposome	575±4.04	150-750	3-4	3.61±0.18	76.75±4.59
Uni lamellarliposome	150±6.66	100-200	1-2	1.19±0.06	84.95±7.53

LENDV=Liposome Encapsulated Newcastle Disease Vaccine.

• Electron microscope; scan and transmission: The samples of liposome 0.5 g, Scanning and transmitted micrograph were done in Abcam Laboratory, USA, for E.M. imaging scan and transmission for clarifies laminar and size of liposome with general surface appearances laser beam scatting technique (Płaczek and Kosela, 2016).

LipoENDV entrapment titer

• The haemagglutination ability of the NDV was used to the estimation of antigen encapsulation inside the prepared liposome, the liposomal vaccine preparation was centrifuged at 3000 round/minute.

• The amount of virus that was unencapsulated in the liposome was measured using a haemagglutination test (HA).

• The difference between the primary HA activity of the virus, before the liposomal incorporation and that present in the supernatant obtained from the liposomal preparation after centrifugation, reflect the amount of incorporated virus (Khalifa *et al.*, 2014).

Entrapment efficiency % =

$$\frac{\text{Totalvaccine} - \text{freevaccine(supernantant)}}{\text{The total amount of vaccine}} \times 100$$

Results

• Microscopic appearance; Light and electron microscope: The liposome lamellar and size were inspected for LipoND$_{Las}$ formulas *via* light phase contrast imaging depict; fig. 1 showed multilamellar multivesicle liposome of MlipoEND$_{Las}$V as well as the UlipoEND$_{Las}$V exhibited unilamellar of individuals liposome fig. 2 with large whole size core.

The electron microscopic imaging displayed obvious multilamellar of MlipoEND$_{Las}$V and single or double layers of MlipoEND$_{Las}$V fig. 1 and uniliposome fig. 2, the topographical distribution revealed to coincided with result, in table 1, lamellar 3-4 and 1-2 of both multi and uni lamellar END vaccine respectively.

• Liposome Standardization : The size of liposome-encapsulated ND vaccine was distinguished in two forms multilamellar and unilamellar liposome have size 575±4.04 and second 150±6.66 respectively, the Entrapping percentage of liposome-encapsulated ND$_{Las}$ vaccine in multilamellar liposome was 76.75±4.59, While in unilamellar liposome was higher than multilamellar reach to 84.95±7.53 as shown in table 1. The size of multilamellar significant $P<0.05$ more than unilamellar and same significant $P<0.05$ forward to Lamellar number range and mean.

Discussion

The results of modified Banghasome presented qualified entrapment percent 84.95±7.53 and 76.75±4.59 in unilamellar and multilamellar respectively. The particle size of UEND150±6.66 and MEND 575±4.04; in aqueous media this share a documented idea the liposome fact built-in entirely enclosed by a membrane phospholipids-Cholesterol, interactions between polar head groups and Van der Waals' interactions that corresponding to spontaneous formation of closed bilayers of lipids (Frezard,

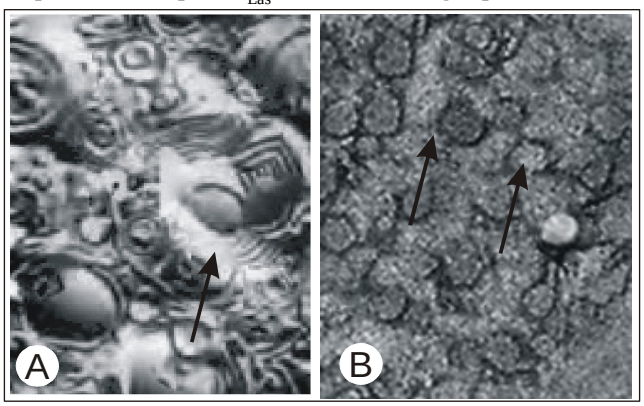

Fig. 3: Electron micrograph of Multiliposome encapsulated ND vaccine set by phosphatidylcholine-cholesterol in Chlorophorm-Methanol 2:1. (A) Transmission depiction characterized by multilamellar multivesicle liposome yellow arrow; (B) Scan depiction categorized by lamellar shape yellow arrow and wide lumen red arrow.

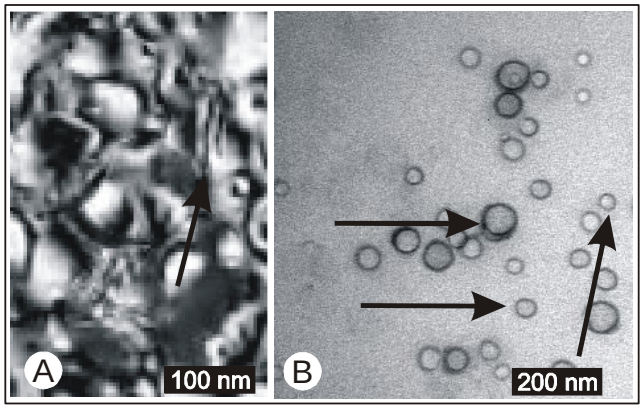

Fig. 4: Electron micrograph of uniliposome encapsulated ND vaccine set by phosphatidylcholone-cholesterol in methanol. (A) Transmission depiction characterized by unilamellar univesicle liposome yellow arrow; (B) Scan depiction categorized by lamellar shape yellow arrow and wide lumen red arrow.

1999; Fan *et al.*, 2012). The entrapment amount of positive correlated with liposome size and negatively with lamellar number (Laouini *et al.*, 2012) according to this fact the increase lamellar number reduced entrapment of vaccine due to narrowing the lumen size of liposome whereas, both size has an encouragement of increase the amount of vaccine entrapment, still approved the increase the amount of entrapment in unilamellar and multilamellar and both were an efficient amount improved dose vaccination in a proper dose.

Liposome can be prepared by entrapped materials both within their aqueous compartment (water-soluble materials) and within the membrane (oil-soluble materials) (Sharma and Sharma, 1997 and Onuigbo *et al*., 2011) and because of unilamellar liposome have a large inner aqueous core, therefore, it is ideal for encapsulation hydrophilic antigen, while multilamellar liposomes have more than one bilayered structure and range from a few to hundreds of nanometer in diameter; they are ideal for encapsulating hydrophobic antigens (Marasini *et al*., 2017). Lipid bilayers numbers of liposomes also influence the encapsulation efficiency and the vaccine release that when liposomes are taken up or processed in the cell, the intracellular fate is affected by the lamellarity (Laouini *et al*., 2012).

The vesicles appeared tightly packed under the transmission electron microscope, indicating that the phosphatidylcholine and cholesterol, which are amphipathic lipids, thermodynamically organized themselves to form stable self-closed vesicles. Generally, the more ordered and hence tightly packed the membrane of a liposome, the less permeable it is (de Gier, 1968; Onuigbo *et al.*, 2012).

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