



CHARACTERIZATION AND STANDARDIZATION OF LIPOSOMAL TYLOSIN PREPARATION

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Abstract

A liposome is considered the most successful new drug delivery system of Nano behavior to improve therapeutic effectiveness, reduced drugs side effects and toxicities and minimized dose administered. The aim of research production of the liposomal tylosin and standardization liposome created from cholesterol by thin-film depressing method, the lipid film hydrated by aqueous phosphate buffer containing tylosin. The liposome entrapment efficiency was 88% multi lamellar and multi vehicles form, with size range nm). The new formula of multi lamellar liposome carrying tylosin was standardized an efficient and positive tolerance to hypo-hypertonic media and pH stability.

Key words: Tylosin, Liposome, *In vitro*, pH, standardization, Nano-Pharmacology.

Introduction

Tylosin, (C₄₆H₇₇NO₁₇) was counted a macrolide antibiotic with an active bacteriostatic against mycoplasma and Gram-positive bacteria e by inhibiting protein synthesis through binding to 50s subunits of bacterial ribosome causes inhibition bacterial growth. Tylosin is commonly used in veterinary therapeutic maneuvers and one of the problems is the residual amount and withdraws time in animal tissue of different animal species. Tylosin physical properties are weak base "pKa 7."73" and Low soluble in an organic solvent (alcohol, esters, ketones, ether and acetone), it is widely in veterinary medicine for different infectious diseases treatment endorsed colitis, mastitis, metritis, arthritis and pneumonia {Mohanad A. AL-Bayati Laith H.AL-Salhi, 2018, Attenuation of Ifosfumide adverse effect on Leyding, sertoli and spermatogonia cells of Ram Testis by Liposome Technique *in vitro*, University of Kufa, faculty of veterinary medicine kufa journal for veterinary medicine sciences. Vol (9) No(2)}. Tylosin has an unwanted effect without awareness of their jeopardy of animal performance, several documented taste.

The pharmacokinetic profile of tylosin was manifested by high absorption in GIT of different animals approximately reached to maximum peak of absorption concentration at 1-2 hours. The Tylosin is liver metabolism

and fast elimination from the blood plasma ranged between (<10 - >70) in different species of animals. The $t_{1/2}$ 0.4 hours, there was positive correlation between body mass and clearance of animals, Several notions in literature share several documents in tylosin adverse effect referred to gastric disturbance, decreased appetite, anorexia and diarrhea (Westermarck *et al.*, 2005).

Reason must be taken care with awareness on jeopardy on animal fitness via unwanted effect, several trials were adapted to improvement tylosine via synergistic combination alternative adjuvant or regimen dose as well as overcome the resistance phenomenon for improving patient outcome and prevent negative consequences of short comes, Nano-pharmacology approved on new technique improved the drug and overcomes undesirable effects, as well as economics, minimized the drug dose (Cleveland, 2017).

The Nano term common approved new approaches for development drug behavior and pharmaceutics via nanotechnology in medical application act one of important drug delivery system for maximized efficacy-potency and minimized the adverse effect, this technology improves the pharmacokinetics of drugs and increase bioavailability by selectively targeted tissues (Jk, 2012).

Nano-liposomes are biodegradable drug delivery system compromised phospholipids; phosphatidylcholine,

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a lamellar form of shell one or more layers, act as Nano-carrier of drugs accepted several advantages of Nanoic traits which are considered as a method to delivery drug system. A liposome is used as an oral dosage to protect the encapsulated drug particles from degradation in the digestive system (Bozzuto *et al.*, 2015; Bangham *et al.*, 1965).

Aim of study Tylosin is one the drug in veterinary communally usage in several cases in different dosage and species especially in poultry, the overcome of tylosin residues in different tissue may be cause-effect in human health (consumer) therefore evaluated the study to reduce the adverse effect {Dr. Iman Rasool Alshati* and Dr. Najlaa S. Ibrahim* 2019, Muco-adhesive Gel of liposomal progesterone and liposomal PMSG vaginal formula characterization and preparation *in vitro* and in situ in vaginal Mucous in Ewe, University of Baghdad/ collage of veterinary medicine/ department of surgery and Obstetric, Vol(18) No(2)}.

Materials and Methods

The technical and practical maneuvers of the experiment were conducted in Laboratory of pharmacology and toxicology in the College of Veterinary Medicine- the University of Baghdad under. The ethical program was base on protocols authority of animal ethical report regulations according to AL-Bayati and Khamas, (2015) as entitled "Standardized guidelines for the Careful Utilize Laboratory Animals in Research of Iraq".

Liposome synthesis

The liposome loaded tylosin was prepared according to Bangham (Marie and Habeeb, 2012) thin-film method followed by five steps.

1. Mixing: Phosphatidylcholine 0.5 g (Chem Cruz Company, Spain, CAT. 28319) was mixed with Cholesterol 0.25 g w/w (Spain CAT. 9651).

2. Dissolving: organic solvent Methanol 10ml (CHEM-LAP, Belgium CAT 268402803) with Chloroform 5ml (India, CDH Cat. No 861117) v/v mixed for 3 minutes and phosphatidylcholine-cholesterol mixture was dissolved in an organic solvent; vortexes for 30 minute at $37 \pm 1^\circ\text{C}$.

3. Evaporation: Organic solvents were evaporated by vacuum pump with vortex 30 minutes 37°C until completely dry and liposomal formation thin film deposited and layered on the tube wall presented empty liposome.

4. Depressing:

• Tylosin working solution preparation 20%: the tylosin 0.2g was dissolved in phosphate buffer 1 ml, pH 7.2 (brook and Russell, 2001). And incubated in water bath 37°C .

• Encapsulation tylosin: tylosin working solution 1ml

was mixed with 0.75 g of empty liposome incubated at $37 \pm 1^\circ\text{C}$ and dispersed for 30 minutes by vortex 2,500 rpm/minute rpm.

5. Entrapment estimation:

• The formula of liposome encapsulation tylosin was centrifuged; 4000 rpm/minute for 30 minutes, the supernatant was checked volume separated. The volume and tylosin concentration were checked and tylosin estimated by spectrophotometer.

• The liposomal-tylosin formula 0.2 g was dissolved in 2 ml methanol vortexes 30 minutes then centrifuged for 30 minutes; the tylosin concentration was checked entrapped tylosin.

The entrapment percent and entrapment efficiency were calculated as the following equation:

$$\text{Tylosin entrapment} = \frac{\text{Amount of tylosin entrapped}}{\text{The total amount of the tylosin}} \times 100$$

$$\text{Tylosin entrapment efficiency} = \frac{\text{Total sylosin} - \text{free tylosin (supernatant)}}{\text{The total amount of tylosin}} \times 100$$

Liposome encapsulated tylosin standardization

The Liposome was challenged several turnovers of stability approved *via* :

1. Electron micrograph: The sample of liposome was kept in the deep freeze -20°C after packing sealed in Eppendorf tube with the 1ml suspension of liposomal tylosin. The liposomal type, lamellar and size has been evaluated by electron microscope scanning and transmitted micrographs were done in Al-Mthafary Lab. Iran (Fennema, 1996 and Placzek and Kosela, 2016).

2. Number of liposomes:

• Absorbance curve and λ Max: The liposome carrying tylosin 25%; 0.5 mg diluted by 2ml normal saline 0.85%, the absorbance curve was plotted by scanned in spectrophotometer 200 to 900 nm (Wittung *et al.*, (1994) and (Tasi *et al.*, and chen, 2003).

• Calibration curve of liposome counts

The liposome stock solution 10%; 0.1 g of the liposome in 1 ml of normal saline was prepared. The serial dilutions were carried and measured the absorbance; the best-fitted line of liposomal dilution versus absorbance was plotted.

The serial solutions were counted the liposome by hemocytometer and calculated as following equation (brook and Russell, 2001)

$$\text{Total liposome per ml} = \frac{\text{Liposome number} \times \text{Dilution factor}}{\text{Squares number} \times \text{Volume of square}}$$

Volume of square: $1\text{mm} \times 1\text{ml} \times 0.1\text{mm}$

Dilution factor: 1/10; Squares number: 5

• The number of liposomes indexed versus absorbance plotted linear regression curve and the liposome concentration per ml was estimated

3. Osmo-tolerance: Spectrophotometric assessment of osmo-tolerance of liposomal tylosin via calculated the liposome survival numbers with the challenge the dilution assay (Bibi and Kaur, 2013). Liposome formulated tylosin 0.25%, 1ml diluted in normal saline NaCl and hypotonic dilutions (5.5, 4.5, 3.5, 2.5, 1.5 and 0%). The number was recorded at 470 nm in a spectrophotometer.

4. pH tolerance: The assessment of liposome carrying tylosin stability in pH changes depended on the direct effect of hydrogen ion on survival numbers of liposomal tylosine Semalty *et al.*, (Semalty *et al.*, 2010). The liposome suspension 0.25% was acidified to alkali media labeled as pH 1, 2, 3, 4, 5, 6, 7 and 8 the liposome concentration was measured in zero and 1 hour by spectrophotometer at 470 nm.

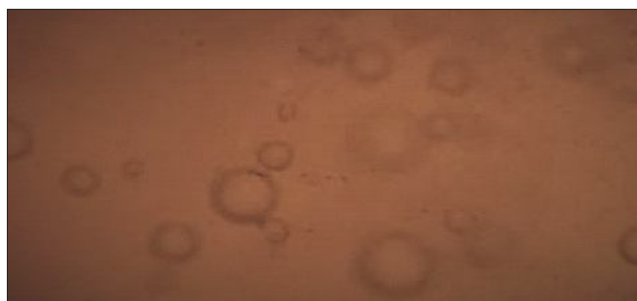
5. In vitro release of tylosin: The releasing of tylosin was estimated according to (Hua-lin and Yu-jiao, 2010) Sheep jejuna strip was layered in oxygenated Petri dish containing stimulant media; NaCl 8.7g, KCl 29.8g, CaCl 5.9 g and glucose 5 g prepared 100ml in normal saline. The liposomal tylosin allocated on the mucosal surface of jejunum; the liposomal formula was not contacted with media. The samples were collecting at a time interval (2, 4, 6, 8, 16, 24, 32), the liberated tylosin estimated was by spectrophotometer.

Results

Liposome Characteristics

• Morphology of Liposome :

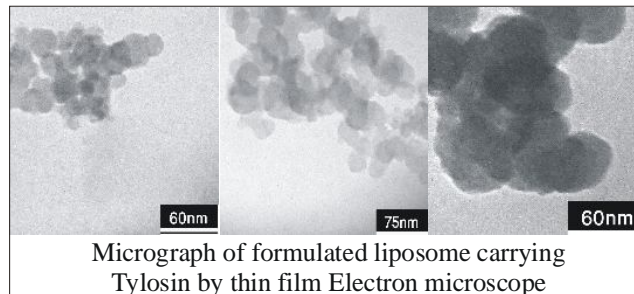
Light microscope: Liposomal Tylosin and empty liposome were examined by the light microscope with magnifications X400 and X1000 for estimation liposome morphology with and without tylosin. The empty liposome showed regular rounded vesicle shape, while, the liposomal tylosin showed heterogeneity in size and shape



Micrograph of formulated liposome carrying Tylosin by thin film light microscope

of the liposome as multi vesicular like grab unit.

Electron microscope morphology: The characteristics of liposomal tylosin and empty liposome showed the multi lamellar and size and diameter Multi lamellar, multi vesicle and different size of liposome denoted by an arrow which appears.



• Calibration curve of liposome counts

The categorized and classification of liposome showed multi lamellar multi vehicle conventional type and liposomal size of carrying tylosin formula was sized diameters 42-96 nm and the size of empty liposome was 44-89 nm table 1. The liposome size entrapped tylosin larger than empty significantly $p \leq 0.05$.

Lamellar of liposome

The lamellae of liposome carrying tylosin and empty liposome were shown in the table 1 there were no significant $p < 0.05$ between empty and entrapped liposome. The mean of lamellae of empty and liposomal tylosin was (3.25-3.61).

• Entrapping efficiency and entrapment percentage

The entrapment percentage and efficiency of liposome carrying tylosin formula as given in table 2 for 10 patches.

Absorbance curve

The result showed that the max peak at 320,364, 472, 616, 764 nm and several located peak cause approved estimation practical opacity.

Osmolarity

The osmotolerance in fig. 2 showed significantly stable in concentration of NaCl at (0.89) on the other side the fact of transient time of osmotolerance of liposome changed NaCl (0.2, 0.5, 0.75, 1) approved some changes per concentration while the liposome was suffering a lower number of liposome in one hour than zero time.

• Effect of pH on the liposomal tylosin

The pH tolerance shows stability at (5-8) exactly but apparently is less stable because the denaturation of proteins content as well as, the zero time is stable more than one hour significantly (0.05).

• Counting of liposome and calibration curve

Liner curve liposomal tylosin in normal saline 0.9% at 37°C standardization stock solution for liposomal tylosin and empty liposome were prepared to get the final concentration (0.25mg/ml) and five serial dilution from stock solution which there was a positive correlation between absorbance and NaCl concentration (0.89) and reflected as well as NaCl concentration display some behavior and considered with absorbance and number of liposome in liner time.

Releasing time of liposomal tylosin

The result to overcome the releasing of tylosin carrying liposome applied on intestinal mucosal strip, the amount of tylosin released was shared a biphasic behavior at the first six hours showed fast released gradually increased value, the 2nd curve behavioral accelerated significantly ($P < 0.05$) at (22-50) hours compared with the first line.

Discussion

Liposome characteristics

• Morphology of Liposome:

Scan and transmission electron microscopy are perform to govern the size and polydispersity (Coldren *et al.*, 2003). However, the electron microscopy has own guide of morphology (Mozafari, 2010). For instance, Electron micrograph provides the liposomal size of the stock prepared liposome. Additionally, the model of measures aggregation of liposomal spheres as vesicle which is seen in the actual size of a solo liposomal sphere shape in which a properly hydrodynamic radius can be detected that tend to clog due to the possibility of electrostatic interactions with the medium which may have a negative charge (Jesorka A. and Orwar O., 2008).

• Size and Lamellar of liposome:

Particle size and lamellarity depend on the method of manufacture Liposomal tylosine chiefly may have an interaction between hydrogen bond and polarity interaction with phosphatidylcholine-cholesterol in the tail part of building unit and generated positive charges of choline part this promote the spherical formation of liposome. All forms of liposome sets formula of Tylosin entrapped liposome of these resulted formulation created the multi lamellar vesicles of liposome there after homogenization of the solution was controlled to maximize the entrapping and lamellar of liposome (Lessieur *et al.*, 1991).

The lamellae of liposome carrying tylosin and empty liposome were shown in the table 1, there were no significant $p < 0.05$ between empty and entrapped liposome.

Range values of liposome lamellar were of both empty liposome and liposomal tylosin. The mean of lamellae of empty and liposomal tylosin was the main size of liposome carrying out of methanol-chloroform solvent method which presumably display the permitted increased stable for lamellar in GIT channel, considering liposome structure displays that the liposome structure MLV and MV medium to highly entrapped from according to homogenized solvent (Lichtenberg and barenhollz., 1988) the average size and the lamellar types of liposome could be influenced by a few factors ; polarity of the solvent of preparation, it was also reported the lamellar size of liposome could modify and liposome (himanshu *et al.*, (2011) Yuchen, F., Qiang, Z. (2013)

Furthermore, the lamellarity of liposomes can be may be formed by interacts with the negative charge on phospholipids of the outer liposomal surface. The interaction outcomes in expansion and lessening of the resonance motion. The pH may affect the method accuracy (Riza M., 1996). Microscopic micrograph including scanning electron microscopy, are used to measure the lamellarity (Ruozi *et al.*, 2011). These display the precise lamellarity, they provide additional evidence such as shape and size.

The lamellarity of tylosine liposomes impacts to an excessive extent the degree of encapsulation efficiency, the efflux rate of liposomal encapsulated tylocin and the chance of a drug afterward cellular uptake. In one study it was witnessed that liposomes organized by the combinations of two lipids large uni-lamellar vesicles preamble proved to be superior to multi lamellar liposome's and dehydration/rehydration liposome's systems to the extent that physical stability was concerned (Huang *et al.*, 2010).

The categorized and classification of liposome showed multi lamellar multi vesicle conventional type and liposomal size of carrying tylosin formula was sized diameters 42-96 nm and the size of empty liposome was 44-89 nm table 1, the liposome size entrapped tylosin larger than empty significantly $p < 0.05$.

Liposome is the delivery systems, because of their biocompatible composition as well as superior efficacy, especially the significant improvement in drug circulation. The instance dramatic evolution the both: (i) Physical appearance: size, shape and lamella (ii) Chemical: pH and osmo-tolerance.

The vesicle size is an acute parameter in believed determining the circulation half-life of liposome and both size and number of bilayers affect the amount of

Table 1: Size and lamella of liposome carrying tylosin and empty liposome.

Means± SE of Empty liposome	Means± SE of Liposome carrying tylosin	Range of Empty liposome	Range of Liposome carrying tylosin	Liposome shape
69.85	87.51	44-89	42-96	Size
3.25	3.61	1-4	1-4	Lamella number
<i>The data presented as mean ± SE; Number of patches 10</i>				

drug encapsulation in the liposome (Akbarzadeh *et al.*, 2013). The current study has shown that the liposome prepared by Bangham ordinary methods size mean was 165 ± 5.82 nm agreed with yaha, (Yaha, 2017) table 1, which was large multi lamellar vesicle (Laouini *et al.*, 2012) Woodle *et al.*, (1992) suggested that the mean particle diameter was increased from 100 to 200 nm leads to shorter elimination half-life of liposome and decreases the plasma protein binding which increases the liposome clearance. The size of liposome permitted stable for lamellar in GIT canal, that liposome structure MLVs and MVs display the high entrapment according to the using of miscible solvent (methanol-chloroform) (Gupta and Suman, 2008). The polarity of the solvent can influence the size and lamellar type of liposome and also could modify the lamellarity property and entrap liposome (Himanshu *et al.*, 2011). Another fact was the preparation of liposome rich with cholesterol derived MLVs and maintained liposome stability in internal body environment that liposome had charged lipid into the liposome surface maintain certain drug through electrostatic bounds (Sercombe *et al.*, 2015). The impact of cholesterol on liposomal clearance was assessed in the present study that cholesterol was required to maintain the stability of liposome in the plasma. Additionally, it may also inhibit protein binding by shielding defects on the surface of liposome (Samad, A. and Sultana Y., Aqil, M., 2007) thereby presumably preventing them from recognition by opsonizing plasma proteins (Laouini *et al.*, 2012) that found reducing the amount of cholesterol lead to a reduction in encapsulation efficiency. This is probably because cholesterol imparts rigidity and stability to the liposome wall and reducing its amount causes lyses and fusion of the liposome which resulted in low encapsulation efficiency (Socaciu *et al.*, 2000).

Liposomal tylosin Entrapping efficiency and Entrapment percentage

Entrapment efficiency is revealing to the quantity of

Table 2: The entrapment of liposome carrying tylosin formula.

Entrapping %	Entrapping efficiency %	Non-entrapment amount ml	Liposome entrapment
60	88	0.47	0.5
<i>The data presented as mean ± SE; Number of patches 10</i>			

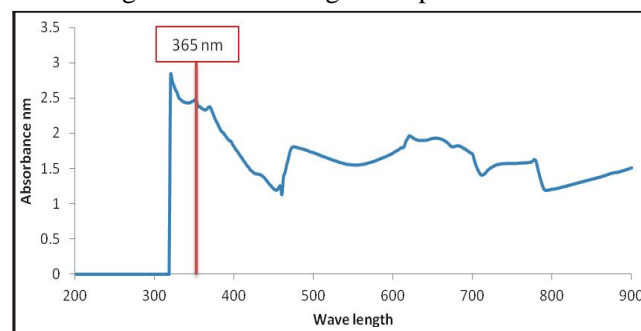
tylocine entrapped in liposomes. It is important as EE can be used to improve the formulation composition prior to reviewing the behavior of the entrapped drugs in physical structure or biological organizations. Tylocine attributed entrapment amount to water-soluble behavior, encapsulation wealth the entrapment within the liposomal core. After elimination of non-entrapped tylosine from the aqueous phase. Entrapment efficiency is commonly clear as the percent portion of entrapped pharmaceuticals to that of the initial concentration used in the liposomal preparation (Ruozi, B., Belletti, D., Tombesi, A., *et al.*, 2011).

The entrapment percentage and efficiency of liposome carrying tylosin formula as given in table 2 for 10 patches. The efficacy of therapeutic molecule is affected by insufficient delivery, accumulation in a specific tissue or unwanted adverse effect even moderate to severe toxicities in non- target organ (Yuchen and Qiang, 2013). Liposome is the delivery system, used to improve the therapeutic effect of the drug by increase its effect kinetically and dynamically. Liposomal size is an important parameter to determine the circulation half-life of liposome and both size and lamella affect the amount of drug encapsulated in a liposome (Akbarzadeh *et al.*, 2013), size of liposomal tylosin prepared by Bangham method mean was 87.51nm agreed with (Yaha, 2017) table 1, which was large multi lamellar vesicle (Laouini *et al.*, 2012).

The prepared liposomal Tylosin has 88% entrapment efficacy table 2 that means good entrapping efficacy due to the solubility of tylosin in the aqueous compartment of liposome.

Absorbance curve and liposome concentration

The result showed fig. 1 that the max peak at 320, 364, 472, 616, 764 nm and several located peak cause approved estimation practical absorbance properties the ÷ max range from 470 nm fig. 2 has proven that maximal

**Fig. 1:** Absorbance curve of liposome carrying tylosin. (*The data presented as mean ± SE; Number of patches 10.*)

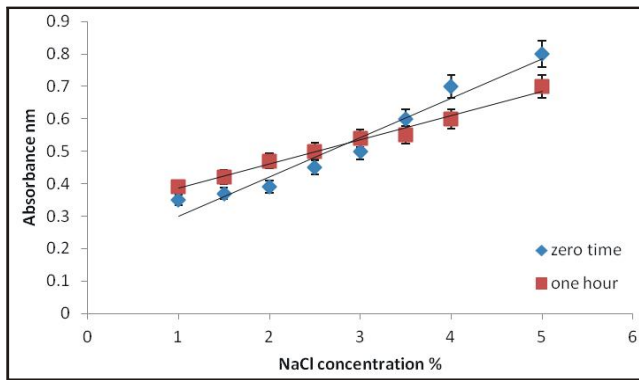


Fig. 2: Effect of osmolarity on liposomal tylosin at zero time and after one hour. (The data presented as mean \pm SE; Number of patches 10).

absorbance peak was 470nm consider the best wave length to record measurement of real sense it showed the highest absorbance values and the highest sensitivity (Wittung *et al.*, 1994). For comparison of absorbance curve result with liposomal Tylosin, Tylosin and empty liposome they have to vary the absorptive and number of entrapped molecules. The spectrum of liposome recorded at 470 nm. The vesicle size facilitated to calculate recording of many peaks nanometer

(Koudelka *et al.*, 2010), the turbidity originated from the empty liposome and liposomal Tylosin was shown 470 nm absorbance was not only depended on concentration but also on partials size (Xu *et al.*, 2007)

Counting of liposome and calibration curve

Liner curve liposomal tylosin in normal saline 0.9% at 37°C standardization stock solution for liposomal tylosin and empty liposome were prepared to get the final concentration (0.25mg/ml) and five serial dilution from stock solution which, there was a positive correlation between absorbance and NaCl concentration (0.85 %) and reflected as well as NaCl concentration display some behavior and considered with absorbance and number of liposome in liner time shown in fig. 4, 5 and 6. For

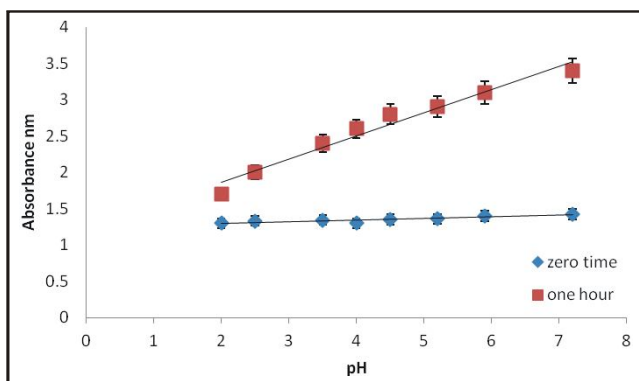


Fig. 3: pH challenge on liposomal tylosin at zero time and after one hour. (The data presented as mean \pm SE; Number of patches 10).

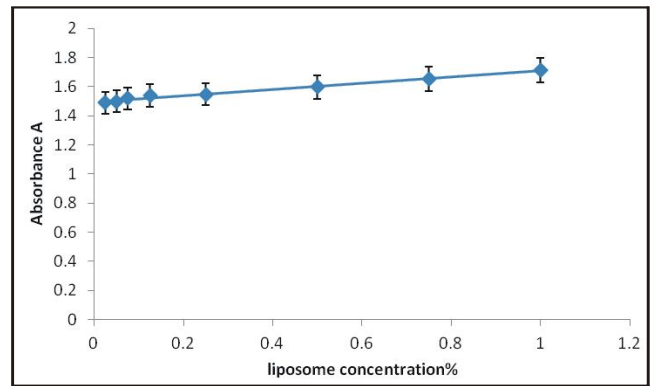


Fig. 4: Spectroscopic estimation of calibration curve of liposome carrying tylosin concentration versus absorbance at 470 nm at 37 °C. (The data presented as mean \pm SE; Number of patches 10).

recovering determination of absorbance by spectrophotometer distinct concentration of liposome and measured aslope of the plot of absorbance 470 nm versus concentration. The absorbance was determined by series prior absorbance to determine the concentration while liposome was calculated by the number per ml using hemocytometer it was depicted versus concentration. Then number of liposome depicted visible spectroscopy absorbance nm to the calibration quantifying. The dose of administered of entrapped per liposome furthermore the cure number and concentration absorbance visible spectroscopy commented and absorbance visible spectroscopy number of liposome hard.

The number of liposome calculated per ml using hemocytometer was depicted versus concentration. The number of liposome depicted visible spectroscopy absorbance nm to at the calibration curve quantifying the dose administered of Tylosin entrapped. The selection of the Bangham ordinary liposome preparation methods to enhance tylosin. The vesicle size facilitated to calculate recording many peaks nanometer (Koudelka *et al.*, 2010). The turbidity originated from empty liposome and liposomal

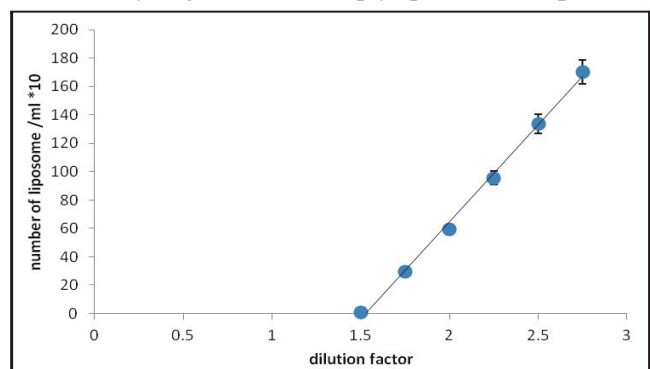


Fig. 5: Spectroscopic estimation of calibration curve of liposome carrying tylosin concentration versus liposome number/ml. (The data presented as mean \pm SE; Number of patches 10).

aspirin was 470 nm absorbance was not only depended on concentration but also on partials size (Xu *et al.*, 2007).

Many bioavailability by decreasing the metabolism of tylosin through first-pass effect in the liver or within intestinal cells by the action of esterase and decrease its side effect on the stomach *via* entrapping to MLVs and MVs which act as a shield to prevent the unwanted effect of tylosin (Bekersky *et al.*, 2002).

MLVs are formed more easily at larger hydrodynamic diameters and thus have greater entrapped volume exhibit a moderate release rate (Marie and Habee., 2012). These may be due overall to the number of a phospholipid bilayer that it has to cross before being released. The morphology of liposome vesicles was shown in fig. 5A and B, using an optical microscope. The images revealed that the suspension contains multi lamellar vesicles (MLVs) and multi vesicular vesicles (MVs) the confirmation of the liposome formation by scan EM was also used. The image in fig. 6 confirms the presence of typical multi lamellar liposome enclosing internal aqueous phase in which the drug is soluble.

Osmo-tonicity and liposome

The osmotolerance in fig. 2 showed significantly stable in concentration of NaCl at (0.89) on the other side the fact of transient time of osmo-tolerance of liposome changed NaCl (0.2, 0.5, 0.75 and 1) approved some changes per concentration while the liposome was suffering a lower number of liposome in one hour than zero time. the osmotic pressure are measured by addition of series concentration of NaCl which was of molecular weight 58.4428 g/mol and has the capability to penetrate phospholipid bilayers (Bordi and Cametti, 2002). The hypo-osmolarity increased the particle size after one hour and increase absorbance curve which may be due to the large particle which undergoes swelling to more than 100

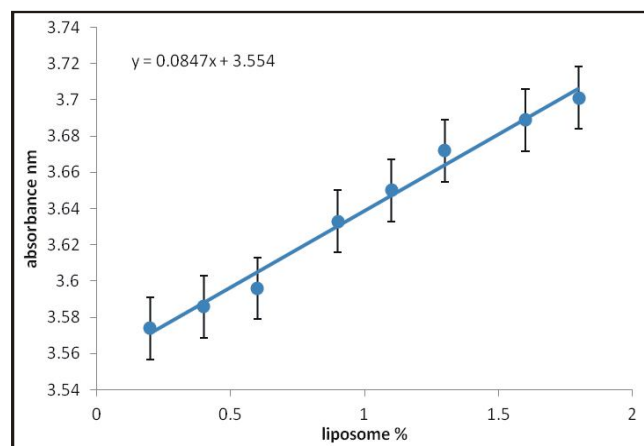


Fig. 6: Stander curve of liposome carrying tylosin. (The data presented as mean \pm SE; Number of patches 10).

nm due to that hypotonic environment causes a net movement of water into the liposome, causing it to swell which gave impression that lecithin and cholesterol had fluidity and elasticity give compliance the increase in their size (AL- Ayed., 2006).

The effect of pH on liposomal Tylosin

The pH tolerance show fig. 3 stability at (5-8) exactly but apparently is less stable because the denaturation of conten. as well as, the zero time is stable more than one hour significantly (0.05).

Liposome is a drug carrier vehicle that can be used to keep advantageous interaction with gastric secretion through oral drug delivery to achieve oral form (Kesisoglou *et al.*, 2005).

For this result showed tolerance in different pH value with less reduction in the absorbance of liposome at pH 2 and 3, fig. 3. The tolerated acidic effect on the liposome stability in oral route after one hour indicated that liposomal tylosin can pass GIT and exert dynamic effect (Chen *et al.*, 2012 and Hua, 2015).

Releasing time of liposomal tylosin

The overcome the releasing of tylosin carrying appear in fig. 7, liposome applied on intestinal mucosal strip, the amount of tylosin released shared a biphasic behavior at the first six hours showed fast released gradually increased value, the 2nd curve behavioral accelerated significantly ($P < 0.05$) at (22-50) hours compared with the first line. For this result, the pharmacokinetic profile of tylosin was manifested by high absorption in GIT of different animals approximately reached to maximum peak of absorption concentration at 1-2 hours. The Tylosin is liver metabolism and fast elimination from the blood plasma ranged between ($<10 - >70$) in different species of animals. The

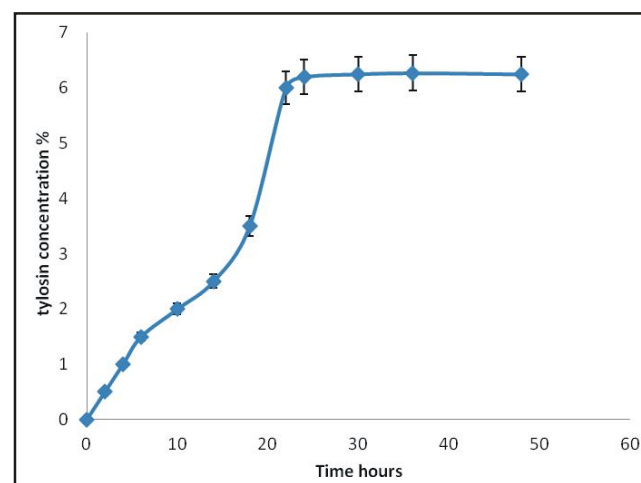


Fig. 7: Releasing time of liposome carrying tylosin. (The data presented as mean \pm SE; Number of patches 10).

$t_{1/2}$ 0.4 hours, there was positive correlation between body mass and clearance of animals, Several notions in literature share several documents in tylosin adverse effect referred to gastric disturbance, decreased appetite, anorexia and diarrhea and may be facilitated to overcome the gastroenteropathic *via* encapsulation tolerance behaviors (Westermarck *et al.*, 2005).

Conclusion

The new formula of multi lamellar liposome carrying Tylosin was prepared with efficient encapsulation and positive tolerance to pH and stability media and and osmotic tolerance. The liposome formulary Tylosin has an impact improved Tylosin *in vitro* kinetically and reflected dynamically as endpoint. As well as promise in future study *in vivo* exploration in animal reduce side effect and determined minimize required doses.

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