

Pharmacokinetics and bioavailability of three promising tilmicosin-loaded lipid nanoparticles in comparison with tilmicosin phosphate following oral administration in broiler chickens

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Abstract: Tilmicosin (TLM) is a semisynthetic antimicrobial agent used mainly in poultry and cattle, but it has relatively poor oral bioavailability. This study was conducted to compare the bioavailability (BA) and main pharmacokinetic (PK) parameters of TLM after oral administration of tilmicosin phosphate (TLM-PH) and three newly prepared lipid nanoparticles (LNPs) of TLM including solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and lipid-core nanocapsules (LNCs). Sixty broiler chickens were divided into eight groups. In four treatment groups (n = 10), each bird was given a single oral dose (20 mg/kg) of a TLM formulation after overnight fasting, and in four control groups (n = 5), the vehicles of those formulations or distilled water were given. Plasma TLM concentrations were analyzed using an HPLC method and the related PK parameters (C_{max} , T_{max} , $AUC_{0-\infty}$, $t_{1/2}$, k_{el} , Cl_B/F , MRT, and V_d/F) were obtained by noncompartmental analysis. The relative bioavailability of TLM-SLNs, TLM-NLCs, and TLM-LNCs were 1.7, 2.7, and 3.6 times, respectively, more than the BA of TLM-PH. Mean C_{max} values were 1.21, 1.58, 1.76, and 2.17 $\mu\text{g/mL}$ for TLM-PH, TLM-SLN, TLM-NLC, and TLM-LNC, respectively. In conclusion, TLM-LNPs improved drug BA and PK parameters, especially the TLM-LNC formulation, which suggests an efficient delivery system for TLM.

Key words: Tilmicosin, lipid nanoparticles, pharmacokinetics, bioavailability, chicken

1. Introduction

Tilmicosin (TLM) is a broad-spectrum macrolide antibiotic derived from tylosin that is used in animals only. It has been approved for the treatment and control of respiratory diseases associated with *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and various bacteria such as *Staphylococcus* spp. and *Pasteurella multocida* in broiler chickens. Generally, gram-negative *Enterobacteriaceae* are resistant to TLM (1–4).

TLM is poorly water-soluble, especially in basic mediums. At present, soluble tilmicosin phosphate (TLM-PH) is used in veterinary medicine as an oral solution but this form has problems of low potency and low bioavailability (BA). High doses of TLM may enhance its efficacy but pose the risk of acute cardiac toxicity since the severity of TLM toxic effects is dose-dependent (5,6). Given these disadvantages, studies on new delivery systems for TLM are warranted.

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Oral administration of drugs is considered the easiest and most practical route but the gastrointestinal (GI) epithelium acts as a physical barrier and may reduce drug absorption and produce poor oral BA. To overcome these problems, a number of new delivery systems have been developed (7,8). Oral BA of drugs is highly affected by their solubility and permeability, the most important physicochemical parameters that determine drug absorption (8). On the other hand, the BA of a drug usually determines its therapeutic efficacy because it may affect the onset, intensity, and duration of action of the drug (1).

Lowering particle size has revealed promise for increasing the dissolution of drugs as well as their BA since it can facilitate the delivery of drugs at the right place and time. Nanoparticle (NP) delivery systems can be prepared using biodegradable materials such as lipids (5). These nanoparticles, which should be stable and nontoxic, can improve the efficacy and safety of loaded drugs (8).

The size and surface properties of NPs highly affect their cellular internalization (9). In recent decades, lipid-based nanoparticles (LNPs) have attracted special interest due to the use of natural or synthetic lipids in their formulation, which demonstrate high drug biocompatibility and controlled release characteristics.

Nanoparticles can augment a drug's absorption by enhancing its dissolution, decreasing gastric emptying rate, and improving drug intestinal permeability. Lipids are recognized as agents that increase lymph formation and encourage lymph flow (10). Basically, the body takes up the lipid and the solubilized drug at the same time. Therefore, it can be considered as a kind of "Trojan horse" effect (11).

Oral LNPs are able to assist in drug dissolution and solubilization because of their ability to protect drugs in a solubilized condition and facilitate their mixing with GI solubilizers such as bile acids. Furthermore, the protective effects of LNPs along with their sustained release properties save drugs from degrading conditions and improve their stability in the GI tract. The nanoscale range of LNPs facilitates their absorption into microfold cells (M cells) of Peyer's patches and eventually into the lymphatic system, thus contributing to bypass the first-pass metabolism (12).

LNPs including solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and lipid-core nanocapsules (LNCs) are colloidal carriers composed of a lipid matrix that is solid or liquid at body temperature. The efficiency of LNPs is highly influenced by their composition; they are usually composed of lipids and surfactants and their structures affect the release properties (13).

SLNs were incorporated as a novel oral drug delivery system in the 1990s. Loading poorly water-soluble drugs into SLNs can improve their GI solubilization, absorption, and BA and provide controlled release properties as well (9,14).

NLCs are considered as the second generation of LNPs and incorporate a biocompatible solid lipid matrix and oily lipid (15). These carriers have demonstrated high BA with various routes of administration such as the oral route (7).

LNCs are a hybrid nanocarrier system with a vesicular structure composed of a polymer and lipid. These nanocapsules have great qualities as drug delivery systems for the oral route and are able to increase the solubility of lipophilic drugs, shield them from enzymatic degradation, enhance drug BA, and decrease the side effects (16,17). In addition, LNCs are more appropriate for prolonged release (18). LNCs are different from other formulations in their composition since LNCs have three main components including the drug, lipid, and polymer. Proper interactions between these components have a vital role in successful manufacturing and efficacy of LNCs (17,19).

The objective of the present study was to perform a pharmacokinetic (PK) analysis and compare the BA of newly designed oral TLM-LNP formulations as potential new delivery systems for TLM in chickens. The main PK parameters of these LNP formulations were compared with the conventional TLM-PH formulation and the possible mechanisms were discussed.

2. Materials and methods

2.1. Chemicals and drug formulations

Tilmosin (tilmicosin phosphate aqueous solution, 250 mg/mL) were provided from Rooyan Darou Pharmaceutical Company (Semnan, Iran) and tilmicosin standard (TLM content 97.3%) was kindly donated by Razak Pharmaceutical Company (Tehran, Iran).

TLM-SLN, TLM-NLC, and TLM-LNC powders were redispersed in distilled water to reach TLM concentrations at 250 mg/mL of TLM. These TLM-LNP formulations were prepared and their physicochemical properties and antibacterial activities were evaluated at the Nanotechnology center of the Faculty of Pharmacy of Tehran University of Medical Science (TUMS). In vitro antibacterial testing was carried out in the Laboratory of Avian Microbiology and Laboratory of Pharmacology of the Faculty of Veterinary Medicine (FVM). The LNP preparations were in nanoscale range with suitable properties as shown in Table 1.

TLM-PH and TLM-LNP formulations were diluted with distilled water to a final concentration of 25 mg/mL prior to oral administration to chickens.

2.2. Experimental animals

Sixty apparently healthy broiler chickens, aged 35 days and weighing 1.0–1.2 kg, were obtained from the poultry farm of the FVM of the University of Tehran. The broilers were housed in cages with a 12-h dark/light cycle. Temperature was maintained at 25 ± 2 °C and humidity at 45%–65% with free access to balanced feed and water. The birds were monitored for 1 week for any apparent clinical signs and adaptation to the study area before administration of the drugs. This study was approved by the ethics committee of the FVM, Project Ethics No. 7506006-6-10.

2.3. Drug administration

The chickens were randomly divided into eight groups, four test groups ($n = 10$) and four control groups ($n = 5$). Birds were fasted for 12 h prior to drug administrations and for 6 h after drug dosing but with free access to water. The test groups were given a single oral dose of 20 mg/kg of TLM-PH and TLM-LNP formulations equivalent to 20 mg/kg of TLM for TLM-SLN, TLM-NLC, and TLM-LNC, respectively. Meanwhile, the control groups received equal volumes of distilled water or SLN, NLC, and LNC vehicles (blanks). Oral administration was done directly

Table 1. Preparation, physicochemical properties, and MIC against *S. aureus* and *E. coli* of TLM-SLN, TLM-NLC, and TLM-LNC formulations and TLM standard.

		TLM standard	TLM-SLN	TLM-NLC	TLM-LNC
Composition		TLM (97.3%)	Hydrogenated castor oil as a solid lipid matrix and polyvinyl alcohol 5% as a surfactant	Compritol 888 ATO as a solid lipid matrix, sesame oil as a liquid oil, and Poloxamer 407 and Tween 80 as surfactants	Eudragit S 100 as polymer, coconut oil as oil core lipid and Span 80 and Tween 80 as surfactants
Entrapment efficiency (%)			66.3 ± 2.67	86.5 ± 2.17	94.0 ± 3.60
Zeta potential (mV)			-15.6 ± 3.21	-23.5 ± 1.13	-16.3 ± 2.51
Particle size (nm)			193.0 ± 2.64	156.6 ± 7.63	116.6 ± 7.63
In vitro release (h) at pH 7.4		97% drug release within 12 h	Initial burst release (18%) within first 2 h followed by a constant sustained release for 120 h	Initial burst release (15%) within first 2 h followed by a constant sustained release for 200 h	Initial burst release (13%) within first 2 h followed by a constant sustained release for 200 h
MIC µg/mL	<i>S. aureus</i>	1	1	0.5	0.5
	<i>E. coli</i>	4	4	2	2

TLM-PH: Conventional tilmicodin phosphate solution; SLN: solid lipid nanoparticles; NLC: nanostructured lipid carriers; LNC: lipid-core nanocapsules.

into the middle of the esophagus using a gavage attached to a syringe following the zero time-point blood sampling.

2.4. Blood sampling

Blood samples (about 1.5 mL) were collected from the brachial or jugular vein into sterile heparinized tubes prior to administration of different formulations (0 h) and at 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h after administration. Within 1 h after sample collection, the blood samples were centrifuged at ~3500 rpm (Eppendorf Centrifuge, Model 5810 R, Germany) for 5 min. The harvested plasma samples were stored at -20 °C until further use.

2.5. Sample preparation

To prepare plasma samples, 50 µL of perchloric acid was added to 950 µL of each chicken plasma sample, vortexed for 30 s, and centrifuged at ~3500 rpm for 5 min. The supernatant was transferred into a special glass tube and 20 µL of each sample was injected into the HPLC system for analysis (1).

2.6. HPLC analysis

TLM concentrations in plasma were measured using an HPLC system (Waters, USA), which consisted of a multisolvent pump, solvent degasser, UV detector, autosampler, interface, and Chromate software. The HPLC column was C18 (5 µm particle size, 125 × 4.6 mm). The modified methods of Clark et al. (20) and Eraslan (3) were used for determination of TLM concentrations in plasma. HPLC analysis was conducted using a mobile phase consisting of 0.2 M ammonium acetate (pH 5), water, acetonitrile, and methanol (20:32:24:24). Mobile phases were filtered under vacuum through a 0.45-µm membrane filter. Chromatographic separation was achieved at a flow rate of 1 mL/min using UV detection at 291 nm (3,20).

A TLM stock solution of 1.0 mg/mL was prepared by adding 10 mg of TLM standard to 10 mL of acetonitrile:water (1:1, v/v). Then it was further diluted in chicken plasma to yield 0.01, 0.05, 0.1, 0.5, 1, and 5 µg/mL.

The HPLC method for TLM in chicken plasma was validated by assessing the linearity, accuracy, precision, recovery, selectivity, and sensitivity according to performance criteria for method validation (3). A standard calibration curve was provided by using six concentrations of TLM (0.01–5 µg/ml) and it was used for calculation of TLM levels in plasma samples.

2.7. Pharmacokinetic analysis

TLM plasma concentration data of each bird were used to depict its concentration-time profile. The maximal plasma concentration of drug (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from the observed concentration versus time profiles. Noncompartmental analysis was used to estimate the PK parameters ($AUC_{0-\infty}$, $t_{1/2}$, V_d/E , Cl_B/E , k_{el} , and MRT). The linear trapezoidal rule was used to calculate areas under concentration-time curves from 0 to 120 h (AUC_{0-120}) and from 120 h to infinity ($AUC_{120-\infty}$) using the following equation: $AUC_{120-\infty} = \text{last } Cp/k_{el}$. The Rel. BA was calculated by using the following equation and PK parameters obtained using Excel 2013.

$$\text{Rel.BA} = \frac{AUC (0-\infty) \text{ of TLM-LNP formulation}}{AUC (0-\infty) \text{ of TLM-PH formulation}}$$

2.8. Statistical analysis

Data were expressed as mean ± SD and analyzed with SPSS 19. The differences in PK parameters were analyzed using one-way ANOVA and Tukey’s test (P < 0.05).

3. Results

The calibration curve for HPLC analysis of TLM was linear over the range of 0.01–5 µg/mL as indicated by $R^2 = 0.999$. The calculated limit of detection (LOD) in chicken plasma was 0.005 µg/mL and the limit of quantification (LOQ) was 0.015 µg/mL. At TLM concentrations of 0.1, 1, and 5 µg/mL, the recovery rates were $99.6 \pm 9.8\%$, $101 \pm 7.5\%$, and $100 \pm 3.7\%$, respectively, and the precision of the method as expressed by RSD% of interday and intraday assays were 4.08, 3.30, and 3.46 and 3.06, 4.05, and 3.74, respectively.

TLM was well tolerated by the chickens without any noticeable events. The major PK parameters and mean concentration-time profiles for TLM-PH, TLM-SLN, TLM-NLC, and TLM-LNC formulations are shown in Table 2 and the Figure, respectively. There was no detectable peak corresponding to TLM retention time in the plasma samples of control groups.

The C_{max} mean value of TLM-LNC and other TLM-LNPs formulation was significantly higher than that of TLM-PH ($P < 0.05$). In general, the mean values of $AUC_{0-\infty}$, $t_{1/2}$, k_{el} , Cl_B/F , V_d/F , and MRT for various TLM-LNP formulations were significantly different from those of TLM-PH ($P < 0.05$). The $AUC_{0-\infty}$ values after oral administration of TLM-LNPs were significantly higher than those of TLM-PH ($P < 0.05$).

4. Discussion

In the present study, we compared TLM-loaded NPs with the conventional formulation, TLM-PH. It was found that all TLM-loaded NPs had significantly higher systemic BA than the TLM-PH.

There are many possible mechanisms for increased oral BA of TLM by using LNP formulations. In general, LNPs are incorporated solid or liquid lipids similar to the fat existing in food. Lipids can stimulate secretion of gastric-pancreatic lipases and colipases. Consequently, according to their residence time, a large amount of ingested lipids are already analyzed in the GI tract (15). The absorption of fatty acids or mono- and diacylglycerides that are available in LNP formulations or made following digestion by GI lipase may facilitate oral absorption of drugs (21). Besides, the release of biliary lipids and salts is stimulated, which in turn enhances the production of mixed micelles, which include solubilized drug molecules. As a result, by participation in mixed micelles, TLM-LNPs could be better absorbed through the lymphatic system (15). The relatively lower absorption of TLM-SLN and TLM-NLC formulations in comparison with TLM-LNCs may be explained by the lower ability of young chickens to digest long-chain fatty acids (C_{14} to C_{24}) existing in hydrogenated castor oil and Compritol 888 ATO. In contrast, the higher BA of the TLM-LNC formulation may be due to the higher affinity of GI lipases in broilers for short- and medium-chain fatty acids (C_6 to C_{12}), which are the main components of its lipid, coconut oil (22).

The oral BA of TLM increased from 1.66-fold in TLM-SLNs to 3.61-fold in TLM-LNCs, which correlated inversely with the particle size of LNPs as it decreased from 193.0 ± 2.64 nm (SLNs) to 116.6 ± 7.63 nm (LNCs). The nanoscale range of LNP formulations leads to a decrease in particle size and highly increases their surface area (15). In addition, the reduction in particle size is positively

Table 2. Pharmacokinetic parameters of TLM in chickens after oral administration of a single dose of TLM-PH, TLM-SLN, TLM-NLC, and TLM-LNC formulations (20 mg/kg body weight). Data are expressed as mean \pm SD (n = 10). Data followed by different letters (a, b, c, d) differ significantly ($P < 0.05$).

PK parameter	TLM-PH	TLM-SLN	TLM-NLC	TLM-LNC
$AUC_{0-\infty}$ (µg h/mL)	43.0 \pm 2.5 d	71.5 \pm 5.8 c	116.4 \pm 17.8 b	155.1 \pm 8.6 a
$t_{1/2\beta}$ (h)	29.3 \pm 2.6 c	39.8 \pm 3.9 b	46.5 \pm 5.0 a	41.3 \pm 3.4 b
V_d/F (L/kg)	20.4 \pm 2.0 a	10.3 \pm 2.7 b	11.9 \pm 1.5 b	7.84 \pm 2.6 b
MRT (h)	42.0 \pm 3.8 b	50.3 \pm 5.8 a	67.2 \pm 8.8 a	59.6 \pm 0.1 a
Cl_B/F (mL/min kg)	0.48 \pm 0.10 a	0.29 \pm 0.04 b	0.19 \pm 0.05 c	0.14 \pm 0.04 d
k_{el} (h ⁻¹)	0.03 \pm 0.01 a	0.03 \pm 0.01 a	0.01 \pm 0.01 c	0.02 \pm 0.01 b
T_{max} (h)	2.40 \pm 0.24 c	5.60 \pm 0.67 b	4.80 \pm 0.80 b	12.00 \pm 0.00 a
C_{max} (µg/mL)	1.21 \pm 0.09 b	1.58 \pm 0.22 a	1.76 \pm 0.38 a	2.17 \pm 0.30 a
Rel. BA (F) %	100	166.2	270.7	360.7

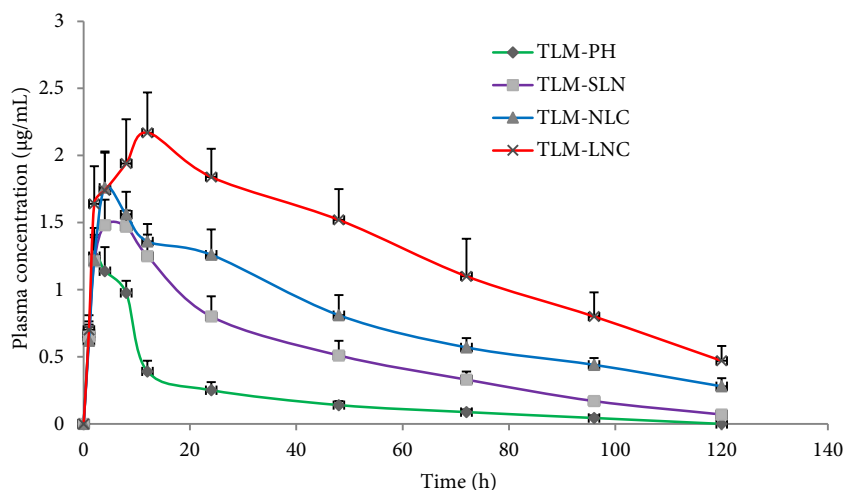


Figure. Plasma concentration vs. time curves of TLM after a single oral administration of TLM-PH, TLM-SLN, TLM-NLC, and TLM-LNC formulations (20 mg/kg B.W.) in broiler chickens. Each value represents the mean and SD (n = 10).

related to an adequate and steady absorption of TLM in the GI tract. In general, the increase in the bioadhesion of these LNP formulations to the GI wall leads to prolong their residence time and their contact with epithelial membranes, which improves their absorption (21). The reduction in particle size may also lead the GI system to uptake them by other routes, such as entry to submucosal tissues through intracellular pathways. The process of GI uptake may include diffusion of particles through mucus and being more accessible to enterocyte surfaces, epithelial interactions, and cellular trafficking (7).

Negative surface charges (zeta potential, ZP) of TLM-LNP formulations can be considered as another possible mechanism for enhancement of TLM oral BA. In general, the glycocalyx renders the intestinal mucosa a negative charge, with which it will attract positively charged nanoparticles. Therefore, the intestinal mobility of particles seems to be highly related to their surface charges, which are inversely related to particle surface charge potentials. With negatively charged particles, higher transport rates can be expected in comparison to near neutral or positively charged particles, whose transport will be highly limited because of particle aggregation and electrostatic adhesive interactions with intestinal mucin fibers. Increasing the efficiency of the penetration through the intestinal mucosa is important to improve oral delivery systems. Nanoparticles should be sufficiently small to avoid severely steric inhibition and adhesion to the intestinal fiber mesh. On the other hand, NPs should have some mucoadhesion to prolong their retention time and contact with intestinal mucosa (8). In addition, the surface charge of NPs also plays an important role in M cell uptake. For instance, it has been reported that negatively charged NPs had higher

M cell uptake than positively charged NPs (23). However, the TLM-NLC formulation did not achieve higher AUC values compared to the TLM-LNC formulation in spite of showing higher ZP values (-23.5 ± 1.13 mV), because the particles size and their surface charges are working together in intestinal absorption processes, and TLM-LNC had more ideal characteristics with regard to its smaller particle size (116.6 ± 7.63 nm) with optimal ZP value (-16.3 ± 2.51 mV).

LNPs can also enhance absorption of TLM through lymphatic flow. They can induce lipoprotein production and lymphatic lipid flux to augment the level of lymphatic TLM transportation, which is significantly affected by the lipid and surfactant types (21). The absorption by M cells of Peyer's patches is an additional way for lymphatic transport of LNPs (12). The small size of LNPs allows for more efficient absorption, particularly in the lymphoid system, consequently bypassing the possible liver first-pass metabolism (19). Although highly lipophilic compounds such as long-chain triglyceride lipids can easily reach systemic circulation by the lymphatic vessels, nevertheless, particle size persists as the most vital factor in lymphatic absorption (24). Particles with larger sizes may last longer in Peyer's patches, whereas smaller NPs are transported directly to the thoracic duct, especially when NPs are coated by polymers, leading them to be easily captured by lymphatic vessels (24). Therefore, it seems that small particle sizes of TLM-LNCs with their polymeric structure are responsible for enhancing lymphatic uptake and increasing the BA.

Furthermore, nonionic surfactants such as Poloxamer 407 and Tween 80 are other factors that may increase the BA of TLM-LNC and TLM-NLC formulations due to

the ability of these surfactants to improve their intestinal permeability by disturbing the cellular membranes and opening the tight junctions of intestinal epithelial cells (15) and facilitating paracellular transportation of LNPs (10). Surfactants can also contribute to the improvement of the affinity between LNPs and the intestinal epithelial membranes, and can enhance their bioadhesion to the GI wall (19). These surfactants are favored for oral formulations and efficiently reduce the degradation of LNPs in the GI tract. The polyethylene oxide (PEO) chains in these surfactants hamper the anchoring of the lipase/colipase complex that is in charge of lipid degradation. By providing sterically stabilizing layers with different thicknesses of PEO chains on LNP surfaces, the in vivo degradation rate of the lipid matrix can be adjusted and slowed down and the LNPs can be given time to be absorbed (13). In addition, Tween 80 can increase intestinal uptake due to its ability to inhibit the p-glycoprotein efflux pump (25). The results of the present study regarding the higher efficiency of LNCs in improvement of PK properties are in line with the findings of Bendera et al., who reported that Tween 80 can be used to deliver drugs efficiently to the brain and to inflamed tissues (16).

The presence of both surfactants, Poloxamer 407 and Tween 80, as ingredients of TLM-LNCs and TLM-NLCs may have contributed to the quality of these formulations, and as a result increased their AUC and C_{\max} values much more than those of TLM-SLN and TLM-PH. These surfactants may act by decreasing the degradation of these formulations, prolonging TLM release and enhancing their crossing through the intestinal barrier.

Indeed, Eudragit S 100, which was used to coat TLM-LNCs, is a polymer with pH-dependent solubility. It releases the drug in GI regions with pH of >7 like the large intestine and colon (8), where it gets gradually soluble. Eudragit S 100 has been used to entrap insulin to protect it from degradation by GI juices and to permit it to be released in GI regions with pH of >7 , where proteolytic enzymes are in low levels (26). Therefore, Eudragit S 100 served as a potential oral carrier in TLM-LNC formulations in the present study. In addition, Mohammadzadeh et al. demonstrated the efficiency of Eudragit S 100 in decreasing p-glycoprotein activity and efflux process. It seems that this polymer improved the BA of TLM by dual actions (27). Generally, the presence of a polymeric coating wall provides a protective layer against the harsh environment of the GI tract such as proteolytic enzymes and may prolong the exposure of TLM-LNCs with intestinal epithelial cells; consequently, it may enhance the BA of TLM (17).

The relatively high BA of TLM-NLCs in the present study is in accordance with the findings of Aburahma and Badr-Eldin, since sustained release with little degradation/aggregation behavior had been demonstrated by using

Compritol 888 ATO because of its long-chain fatty acids (23,28). On the other hand, Severino et al. reported that medium-chain triglycerides lipids are more effective than long-chain triglycerides with regard to sustained release (11), which seems closer to the results of the present study, especially with regard to TLM-LNCs, in which coconut oil constitutes its oily core.

Although hydrogenated castor oil in TLM-SLN was an effective nanoparticle system for controlled release and improvement of PK characteristics of loaded drugs (14), it achieved the least optimal PK parameters values. This may be due to its more rapid degradation in the GI tract, which leads to an increased rate of TLM release (6). Many other factors may have also contributed to decreased TLM-SLN absorption, such as their relatively higher particles size (193.0 ± 2.64 nm) and lower ZP (-15.6 ± 3.21 mV). However, Han et al. (6) suggested that the high initial release rate of TLM from SLN might be helpful because it reaches a therapeutic level quickly.

The TLM release by TLM-LNPs was slowed down just to reach therapeutic serum levels so that the blood concentrations did not reach toxic levels and this obviated adverse effects such as cardiotoxicity induced by high doses of conventional TLM (6). Using TLM-LNPs decreased the TLM plasma elimination rate, as indicated by higher elimination of $t_{1/2}$ and increased MRT, which caused a longer stay for TLM in blood circulation.

Abu-Basha et al. also studied the BA and PK parameters of TLM in chickens using Provital orally at 30 mg/kg B.W. The average AUC_{0-72} was 24.2 ± 3.9 $\mu\text{g h/mL}$, C_{\max} was 2.09 ± 0.37 $\mu\text{g/mL}$, and T_{\max} was 3.99 ± 0.84 h. In spite of using a 1.5-fold higher dose, the values reported for PK parameters were much lower than those of the TLM-LNC formulation, especially with regard to AUC values (1).

Keleş et al. also investigated the PK and tissue concentrations of TLM after oral administration of a single dose (50 mg/kg, B.W.) in fowl. TLM was slowly eliminated from the serum and lungs with $t_{1/2}$ of 30.2 ± 2.4 and 75.7 ± 3.7 h, respectively. The mean C_{\max} was 6.2 times greater in the lungs (7.96 ± 0.30 $\mu\text{g/mL}$) than that in serum (1.28 ± 0.04 $\mu\text{g/mL}$) with T_{\max} at 17.78 ± 7.51 h and 4.66 ± 2.0 respectively (29).

It is expected that TLM-LNCs followed by TLM-NLCs can demonstrate better in vivo antibacterial activity due to their higher AUC, C_{\max} , and sustained release properties (14). In spite of their sub-MIC plasma concentrations against *E. coli*, it seems that TLM-LNPs may be more active than TLM-PH, since the clinical efficacy of TLM formulations not only was affected by plasma drug levels but also was related to intracellular TLM penetration, which usually tends to be more accumulated within avian phagocytic cells (30), like macrophages, monocytes, and heterophils. Furthermore, TLM has high postantibiotic

and sub-MIC effects, which could slow disease development by allowing the immune system to eliminate bacterial infection (30). In general, TLM C_{max} values of all tested formulations detected in chicken plasma after oral administration were higher than the MIC for *M. gallisepticum* and *M. synoviae* (0.0125–0.1 µg/mL). These values were also higher than the MIC of plasma TLM in cattle against *Corynebacterium pyogenes* (0.04 µg/mL) and *S. aureus* (0.78 µg/mL) (1). Consequently, the clinical efficacy of tested TLM-LNP formulations is expected to be highly satisfactory with the TLM-LNC formulation due to their better PK parameters, followed by TLM-NLC and TLM-SLN formulations.

In conclusion, the systemic BA of TLM was significantly enhanced by using oral LNPs (TLM-SLNs, TLM-NLCs, and TLM-LNCs) in comparison to conventional TLM-PH in broiler chickens. Newly formulated TLM-LNPs increased the $AUC_{0-\infty}$, C_{max} , T_{max} , and MRT values

depending on their particle size, particle surface charges, and lipid and surfactant compositions. The best BA results were achieved by the LNC formulation (3.6-fold), followed by NLCs (2.71-fold), compared to TLM-PH. It seems that the hybrid delivery system (LNCs) is more promising to achieve a sustained release TLM formulation with higher antibacterial activity and lower drug toxicity.

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