ORIGINAL ARTICLES

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Nonionic microemulsions for oral and transdermal delivery of gentamicin

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Gentamicin sulfate (GS) is used usually as intravenous or intramuscular solution because of its poor oral bioavailability. However, the intravenous administration is associated with pain and needs some skills. In this study, five nonionic microemulsions (MEs) for oral and transdermal application were developed using nonionic surfactants. The MEs were characterized for their droplets sizes and rheological properties. Moreover, GS encapsulation in the MEs was studied using Fourier Transform Infrared Spectroscopy (FTIR). The transdermal release was evaluated through rat's skin using Franz diffusion cell. Furthermore, one of these formulations was chosen for oral bioavailability studies in rats in comparison to an aqueous solution of GS. These MEs complied with the colloidal properties. Also, FTIR was used successfully to prove the encapsulation of GS and alignment of the surrounding surfactants in the MEs. The best transdermal flux of MEs was 1.892 mg/cm^2*h. The same ME showed a relative bioavailability of 239.7 % in comparison to the oral solution.

1. Introduction

Gentamicin is an aminoglycoside antibiotic produced by Micromonospora purpurea and related species (Meenavilli 2008). It is effective against many aerobic Gram-negative organisms and staphylococci. Moreover, it has low levels of resistance against common nosocomial pathogens and rapid concentration-dependent bactericidal activity and post-antibiotic effect. Furthermore, it is used in combination therapy of life-threatening and multidrug-resistant infections (Nicolau et al. 1995; Gilbert 1997; Freeman et al. 1997; Bertino and Rotschafer 1997; Zembower et al. 1998).

Gentamicin is freely soluble in water and moderately soluble in methanol and ethanol (European Pharmacopeia 2003; Maryadele 2013). Hence, it is very poorly absorbed from the gastrointestinal tract (GIT) and is unstable in acidic pH of the stomach. It is commonly administered intramuscularly and intravenously for being cationic drug which affects its penetration through the mucosal walls of the GIT (Jia et al. 2008; Umeyor 2012).

Transdermal application can improve the therapeutic efficacy and safety of the drugs because of reduced fluctuation of drug in the plasma (Shingade et al. 2012; Schoellhammer et al. 2014). However, the stratum corneum is a barrier for transdermal of hydrophilic drugs like GS (Bouwstra et al. 2003). Microemulsions (MEs) have been studied for transdermal as well as for oral administration because of their good rheological properties and high penetration enhancing capacity. Microemulsions are colloidal thermodynamically stable oil in water or water in oil systems formulated by surfactants and cosurfactants (Attwood 1994; Heuschkel et al. 2008; Hathout et al. 2010; Neubert 2011).

A few studies were designed to assessing and evaluating formulations of alternative routes such as transdermal application or for improving the oral bioavailability of gentamicin. Nnamani et al. formulated patches of gentamicin by solvent evaporation technique using PURASORB® polymers. The patches showed a high permeation flux of 5.161 μg/cm^2 h. and a permeation coefficient of 1.032×10^{-6} cm/h through rat's skin. Moreovers, the formulation revealed good drug encapsulation, stability, tolerability and physicochemical properties (Nnamani et al. 2013).

In a study of the absorption of gentamicin from the GI tract of rats, gentamicin was formulated in a microemulsion using Labrasol. This formulation showed absolute bioavailability (BA) of 54.2% (Hu et al. 2001).

In another study, gentamicin was intercalated into novel PEGylated solidified reverse micellar solutions (SRMS) based solid lipid microparticles (SLMs). SLMs containing 2% w/w SRMS, 3% w/w gentamicin and PEG 4000 intercalated the highest amount of gentamicin and showed a permeation flux of 5.239 μg/cm^2*min and a permeation coefficient of 1.781×10^{-6} cm/min within 420 min (Kenechukwu et al. 2015).

It is commonly suggested that an adsorbent system is useful as an oral solid delivery system of poorly absorbable drugs such as GS when dispersed with a surfactant for the self-microemulsifying drug delivery system (SMEDDS), PEG-8 caprylic/capric glycerides (Labrasol) and the mixture was solidified with several kinds of adsorbents (Ito et al. 2005).

This study aimed to develop and characterize nonionic microemulsions containing GS as colloidal drug delivery systems as an attempt to improve its transdermal and oral bioavailability.

2. Investigations and results

2.1. Three phase diagrams

The first two three phase diagrams of MEs were established for MEs composed of IPM, water and a mixture of Span 20 : Tween 80 (2:3) without GS (Fig. 1A) and with 100 mg GS (Fig. 1B). The second two three phase diagrams of Fig. 1 C and D were for MEs composed of IPM, water:DMSO (0.77: 0.23) and Span 20 without and with 100 mg GS respectively.

The area of clear MEs of first system (Fig. 1 A) extended after the addition of GS to fractions of water between 0.1-0.4 and fractions of surfactant between 0.2-0.3. In case of the second system (Fig. 1 C, D) the shifting in the MEs area was observed to fractions of hydrophilic phase between 0.2 - 0.3 and Span 20 fractions between 0.5-0.8. In both of the two systems MEs region extended towards increasing water fractions and less fractions of Span 20 and Tween 80 in the first system and high fractions of Span 20 in case of the second system.

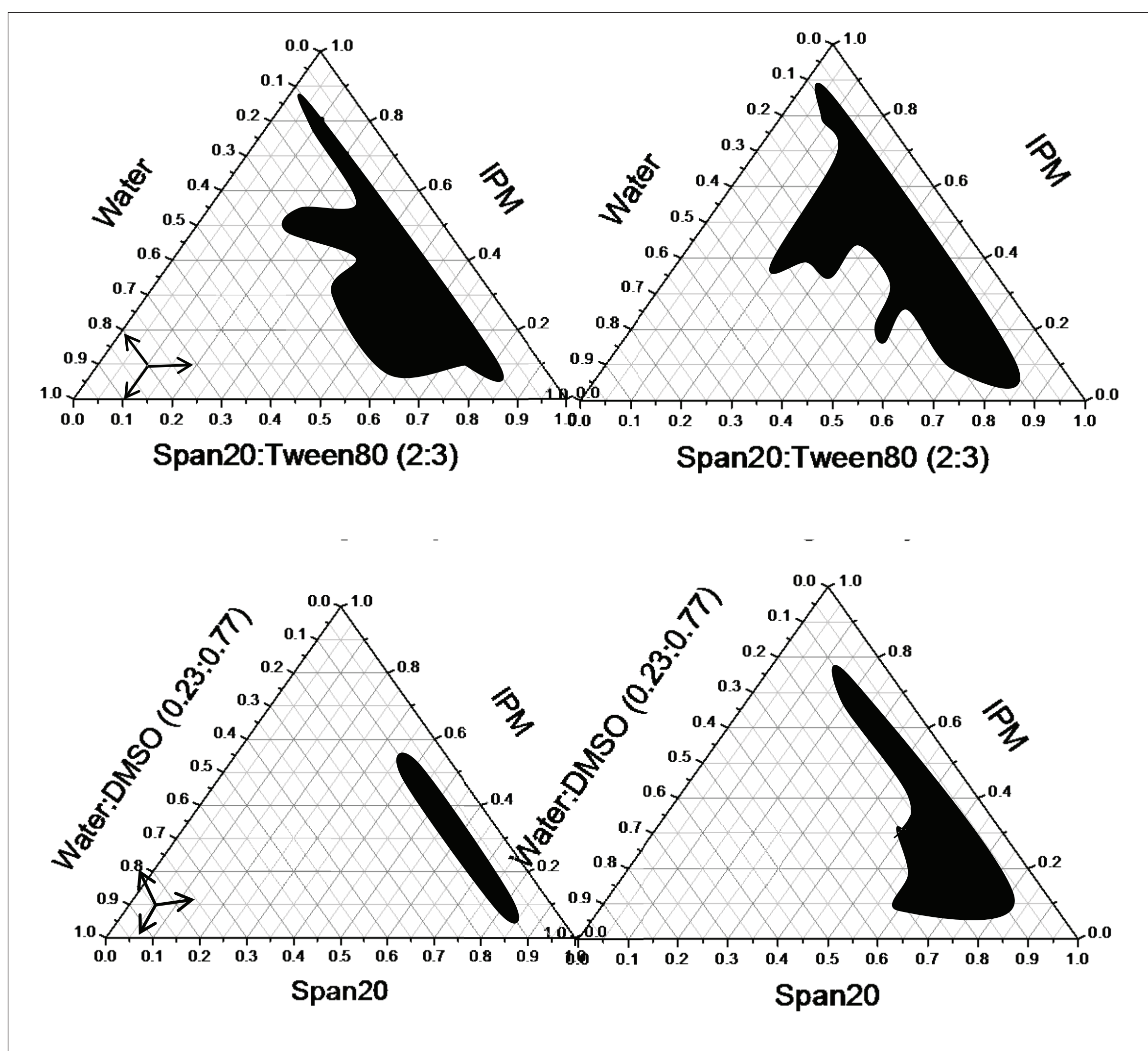

Fig. 1: The three phase diagrams of microemulsions (MEs) composed of IPM, water and a mixture of Span 20:Tween 80 (2:3) without GS(A), with 100 mg GS (B), IPM, water:DMSO (0.77: 0.23) and Span 20 without GS (C) with 100 mg GS (D).

2.2. *Rheological properties*

The rheological properties were determined using a bob and cup instrument with increased shear rate of the different systems and the results are represented in Fig. 2.

The different systems with GS showed lower viscosity than MEs free GS. The viscosity showed some reduction at low rate then showed a very slowly increment. Furthermore, the relationship between the rate of share and shear stress represents straight lines with some nonlinearity. However, the linearity increased with increased GS concentration. Consequently, all the systems exhibited ideal viscosity or Newtonian with weak pseudoplastic characteristics.

2.3. *Droplet size measurement*

zeta-sizer was used to determine the droplets size of the different systems. Moreover, the droplets size of MEs with and without GS was measured to relate the droplet size on both of encapsulated GS and consumed surfactant amounts (Table 4). The droplets size, zeta potential and poly disparity index are listed in table 1.

The droplet size of different formulations complied with colloidal characteristics. The increment in the hydrophilic phase from 0.5 to 1 ml for system s600 and m600 without GS did not lead to increase in droplet size. However, the consumed surfactant amounts were higher in m600 than in system s600. The encapsulation of GS increased the droplet size and decreased the polydispersity index (PDI) in comparison to MEs free GS. The droplets sizes decreased where the PDI increased with increasing the GS from 600 to 1000 to 1200 mg. The stabilized ME using only Span 20 had lower droplet sizes and higher PDI than stabilized MEs using both of

Table 1: Droplet size, polydispersity index (PDI) and zeta potential of different formulated microemulsions with and without gentamicin sulfate

ME	Droplet size			PDI	Zeta potential
	Nm	Vol %	Width		
s600mgs+t without GS	0.6360	10.00	0.2658	3.5600	20.4200
	0.1493	90.00	0.1434		
s600mgs+ t with GS	0.4330	40.70	0.2179	1.3500	0.6200
	0.1424	59.30	0.1351		
m600mgs+t without GS	0.6110	52.70	0.0821	2.1180	0.5800
	0.1384	47.30	0.4490		
m600mgs+t with GS	0.5320	100.00	0.2436	0.1756	2.2100
n600mgDMSO without GS	0.2064	1.70	0.0421	2.9800	0.5700
	0.0232	71.20	0.0157		
	0.0025	27.10	0.0016		
n600mgDMSO with GS	0.6120	59.60	0.0823	5.1500	4.3600
	0.5040	40.40	0.4950		
b1000mgs+t	0.2330	81.50	0.1453	1.1250	0.6100
	0.0617	18.50	0.0313		
d1200mgs+t	0.1209	100.00	0.2219	1.4010	0.6200

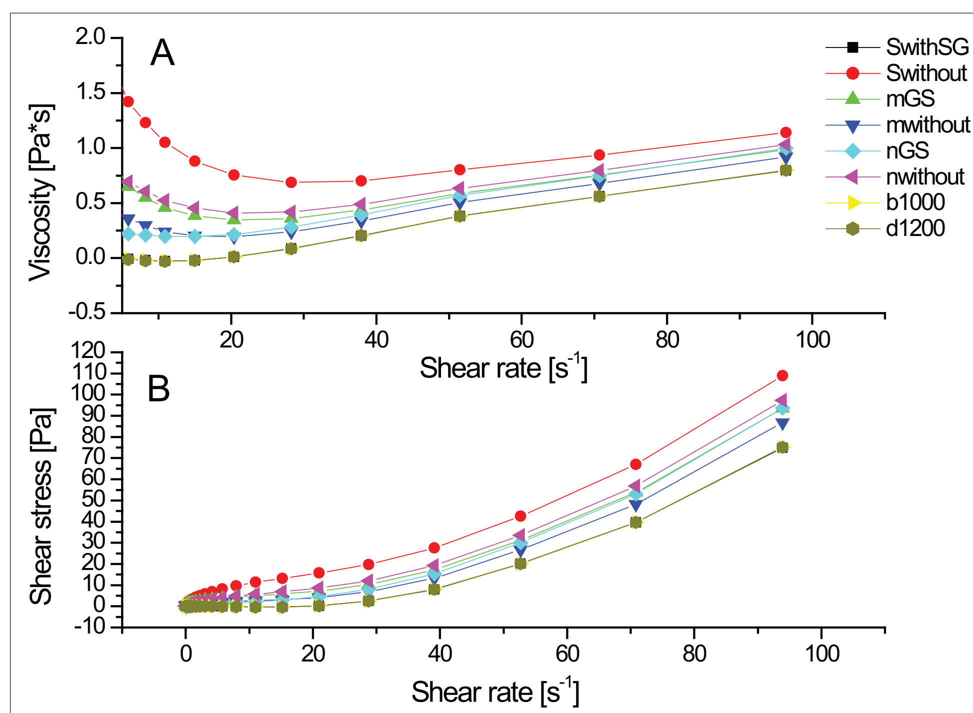

Fig. 2: Rheograms of different developed microemulsions (MEs): The viscosities against shear rate (A), the shear stress against shear rate (B).

Span 20 and Tween 80. Furthermore, systems m600 and b1200 were monodispersed systems with low PDI.

2.4. *Studying of GS encapsulation using Fourier Transform Infrared Spectroscopy (FTIR)*

FTIR was used for studying the structure of MEs. In Fig. 3 A and B the spectra of each single pure ingredient used in producing the MEs and spectrum of ME with and without GS are represented together.

The spectrum of ME was similar to IPM-spectrum which represents the outer phase of the ME. However some new bands were observed in ME-spectrum not found in IPM-spectrum. A broad band between 3100-3671cm^{-1}, small band at 1064 cm^{-1}, shoulder 1033 cm^{-1}, and band between 830 and 450 cm^{-1}. These bands were observed in Span 20 and Tween 80 spectra. The bands between 3100-3671 cm^{-1} more likely belongs to OH, C-H stretching and bending. Moreovers the sharp band 1092 cm^{-1} in Tween 80 and 1088 cm^{-1} of Span 20 which belong to C-O stretching disappeared to appear weaker bands of IPM at 1118 and 1189 cm^{-1} in ME spec-

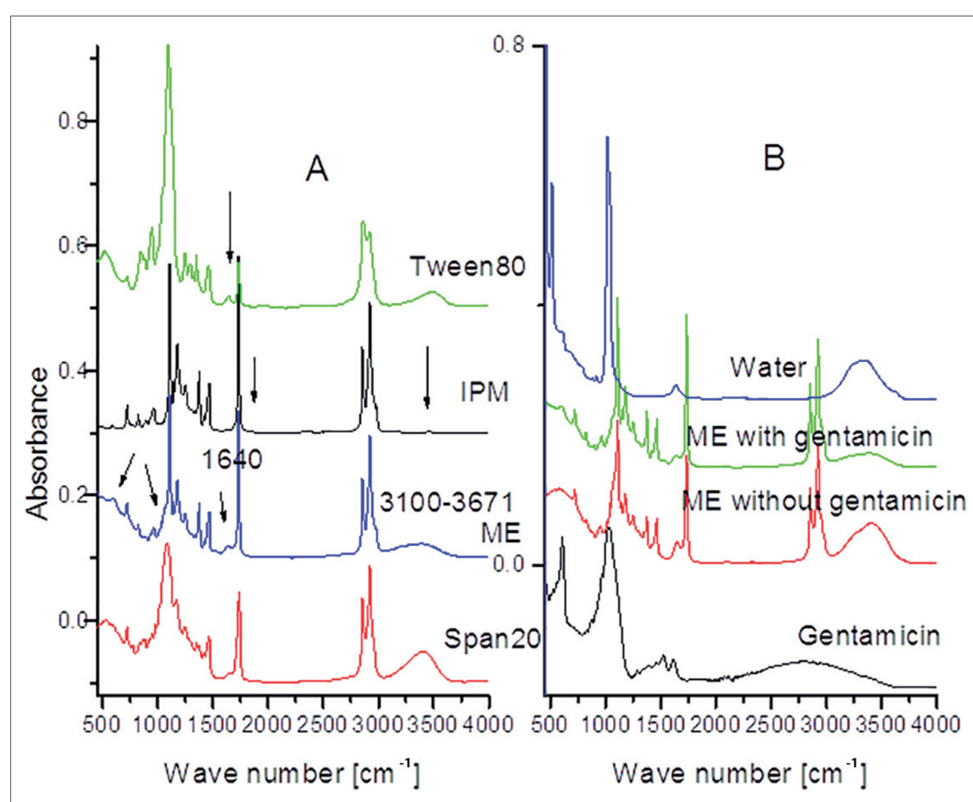

Fig. 3: FTIR spectra for each single pure ingredient were used in producing the microemulsions (A), ME with GS, ME without GS, GS, and water (B).

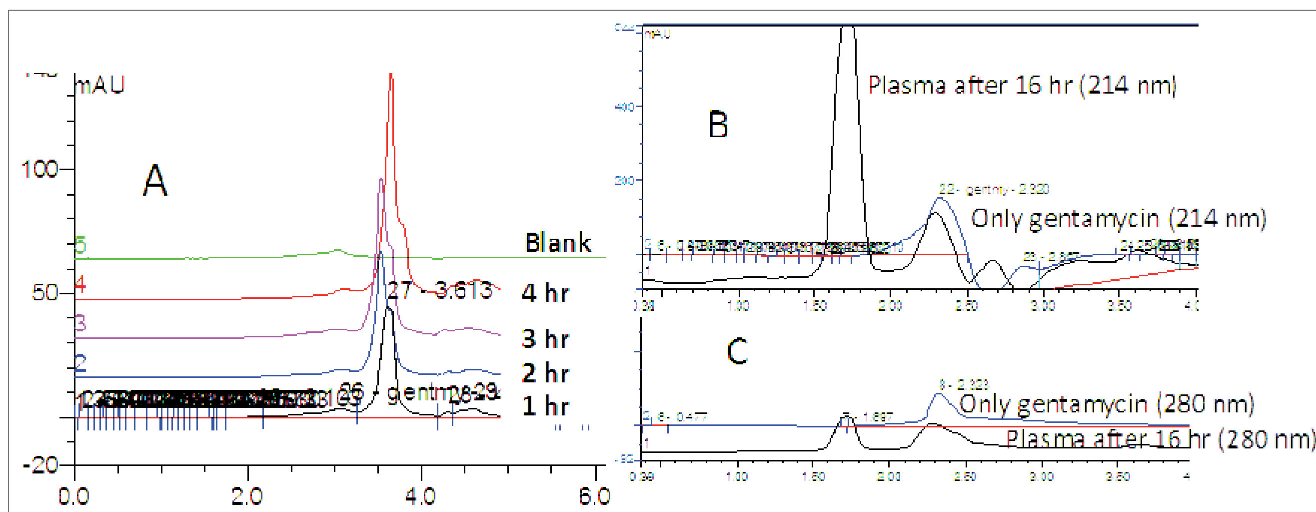

Fig. 4: HPLC chromatograms of gentamicin sulfate (GS) for samples collected from Franz diffusion cells (20 µl injection volume, C18 (4.6*250 mm) column system, mobile phase of water : methanol with ratio of 70:30, flow rate of 1 ml/min and detected at wave length of 280 nm) (A), GS in plasma samples (20 µl injection volume, C18 (4.6*250 mm) column system, mobile phase of water:methanol with ratio of 70:30 and 0.4 ml acetic acid, flow rate of 1.5 ml/min and detected at wave length of 214 nm) (B), as in (B) but at wave length of 280 nm (C).

trum. Furthermore, the band between 450 and 830 cm^{-1} observed in ME, Tween 80 and Span 20 spectra more likely belongs to C-C and C-H bending. We conclude that sorbitol ring and ester bond in Tween 80 and Span 20 oriented to inside the hydrophilic phase where carbon chains of the side groups oriented to outer surface of the droplets in the outer phase.

For testing gentamicin encapsulation in MEs the spectra of ME with GS, ME without GS, GS and water were measured and are represented in Fig. 3B. No difference was detected between ME spectra with and without gentamicin. Furthermore the bands of gentamicin and water did not appear in the spectrum for ME with gentamicin. This indicates that gentamicin was surrounded by surfactant in the inner phase.

2.5. HPLC method

Two calibration curves of the area under the curve against the concentration were plotted. The first one was for quantifying GS in the acceptor of Franz diffusion cell using mentioned conditions in chapter 4.10. This plotted calibration curve was for concentration from 1 to 10 mg/ml of GS which exhibited linearity (R) of 0.997 and a regression standard deviation of 0.72. The second calibration was for concentrations between 0.25 and 2.5 mg/ml for determining GS in rat's plasma which showed linearity of 0.998 and standard calibration curve of 0.32. The limit of quantification (LOQ) according to the European Pharmacopeia (EP) using both two approaches was much less than least measured concentration (The ratio of high of signal of lowest concentration to the noise was much higher 10). The recovery of GS from the MEs using both conditions was more than 98 %.

2.6. Transdermal delivery

Two of the formulated MEs containing different water content (0.5 and 1 ml) but with similar GS content (600 mg) and three other MEs have similar water content (1 ml) but with increased GS content (600, 1000 and 1200 mg) were studied. Moreover, the effect of addition of DMSO to the formulation as penetration enhancer was investigated (table 1).

The dermal penetrability of GS through shaved rat's skin was monitored using a Franz diffusion cell over 24 h. The penetrated GS was collected by removing 1ml periodically from the acceptor medium. The samples were analyzed for determining GS using HPLC (Fig. 4A). The removed GS in each sample was calculated and added to the subsequently estimated amount.

Penetrated GS amounts per cm^2 were measured over 24 h and the cumulative amount per cm^2 plotted against the time. The penetration profiles of different formulations are presented in Fig. 5.

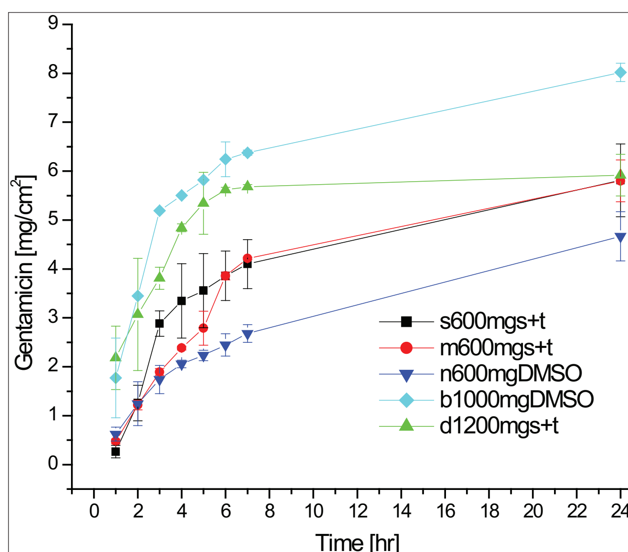

Fig. 5: Cumulative penetrated gentamicin sulfate (GS) amounts per cm^2 using deferent formulations against the time per hour (h).

The Flux (J_{ss}) was evaluated from the slope of the line at steady state (Eq. 1). Also, the lag time (t_{lag}) was estimated from intersect with time axis. Furthermore, the permeably constant was calculated using Eq. 3. The results are represented in Table 2.

The flux of formulation s600mgs+t decreased by one half with increasing the hydrophilic phase from 0.5 ml to 1 ml in system m600mgs+t. However, using DMSO in system n600 did not lead to an increase in flux. Furthermore, the increase of GS content from 600 mg to 1000 mg in ME b1000mgs+t could increase the flux to 1.892 mg/cm^2*h. Moreover, a further increase in the encapsulated amount of GS to 1200 mg in case system d1200 reduced the flux of GS in comparison to system b1000.

Table 2: Estimated flux, lag time (tlag) and permeability constant of different formulated microemulsions (MEs) through rat's skin using Franz diffusion cell

	Flux (Jss) mg/cm²*h	t_{lag} (h)	Cs mg/ml	Kp=Flux/Cv (cm/h)*10⁻³
s600mgs+t	1.282	0.86	142.86	0.008974
m600mgs+t	0.654	0.27	65.93	0.00992
n600mgDMSO	0.474	0	65.93	0.007189
b1000mgDMSO	1.892	0.025	151.5	0.012488
d1200mgs+t	0.946	0	230.5	0.004104

2.7. Oral bioavailability of GS:

System b1000 was chosen to be tested for oral bioavailability in rats in comparison to GS solution in water. The absorbed amounts of GS were monitored in plasma using HPLC over 25 h (Fig. 4B). The plasma level versus time curve was plotted over 25 h (Fig. 5A and B) and the area under curve was calculated using phoenix program.

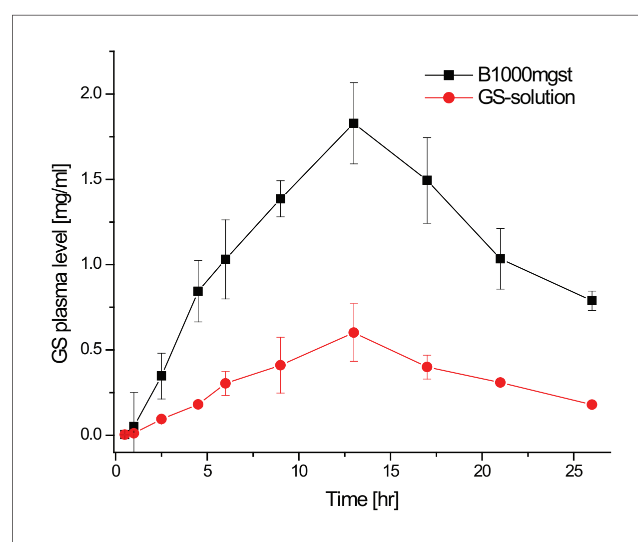

Fig. 6: Plasma level time curves of microemulsion b1000 and of gentamycin sulfate solution.

Furthermore, the absorption rate constant (K01), elimination rate constant (K10), maximum concentration (C_{max}), and time of maximum concentration (t_{max}) were estimated for the solution and ME by applying one compartment open model, first order input with lag of time by using phoenix program. The results are given in Table 3.

Table 3: Estimated pharmacokinetic parameters of gentamicin sulfate in b1000 microemulsion and aqueous solution: area under curve (AUC), the absorption rate constant (K01), elimination rate constant (K10), lag time (tlag), maximum concentration (Cmax) and time of maximum concentration (tmax).

pharmacokinetic Parameter	Units	Solution	Microemulsion b1000mgst
AUC	h*mg/ml	11.51±4.16	41.38±11.89
K01	1/h	0.109±69.65	0.103±17.98
K10	1/h	0.109±69.83	0.102±17.90
Tlag	h	2.22±0.59	1.85±0.53
T_{max}	h	11.36±4.27	11.63±0.79
C_{max}	mg/ml	0.463±0.044	1.557±0.1

The estimated percentage relative bioavailability (PRBA) (eq. 4) of b1000 ME in comparison to the solution was 239.7 %. However, GS shows a slow rate of absorption and absorption rate constant was nearly equal to the elimination rate constant in both systems. As a result, the time of maximum concentration was observed too late after 11.5 h for both formulations. Also a lag time was estimated for ME of 1.85±0.53 h where was in case of the solution 2.22±0.59 h.

3. Discussion

The results show that the developed systems comply with colloidal properties. However, The observed extension in the region of clear MEs in the three phase diagram of both two systems towards increasing water fractions after GS addition may be related to decreased free water which increases the ability of MEs to uptake water. GS is a hydrophilic drug and can form hydrogen bonds with water molecules which reduce the cohesive forces between water molecules and change interfacial tension the hydrophilic phase which affects the consumed surfactants to produce clear MEs.
Furthermore, the detected weak peseudoplasic viscosity which was accompanied with ideal viscosity for MEs may be the results of decreased solubility of IPM and of an interaction between hydrophilic and lipophilic phases. This comes with our observation during the development of MEs that the MEs had transparent gel structure at low fractions of IPM. These MEs-gels transformed to fluid after increasing IPM from 2 to 3 ml. These low peseudoplasic characteristics may because these MEs retained some characteristic of the gel structure. Moreover, this effect is reduced with increasing GS content in the case of 1000 and 1200 mg and may be due to reducing free water with increasing GS content as mentioned before which reduced the interaction between the hydrophilic and lipophilic phases.
The increment in the hydrophilic phase from 0.5 to 1 ml for systems s600 and m600 without GS did not lead to increased droplet size. However, the consumed surfactant amounts were higher in m600 than in system s600. The encapsulation of GS increased the droplet size and decreased the poly dispersity index (PDI) in comparison to MEs free GS. The increase in droplets sizes decreased the interfacial area which led to reduced in consumed surfactant for stabilizing the ME. The droplet size decreased where the PDI increased with increasing the GS from 600 to 1000 to 1200 mg. The stabilized ME using only Span 20 had lower droplets sizes and higher PDI than of stabilized MEs using both of Span 20 and Tween 80 which can be related to the small structure of Span 20 comparing to the structure of Tween 80 and to the increased stability of MEs in case of suing combination of two surfactants. The encapsulation of GS increased the droplet size and decrease PDI in general. Systems m600 and b1200 were monodispersed systems with low PDI. FTIR proved that dissolved GS in water encapsulated inside the water droplets surrounded by surfactant which is an important factor to enhance the penetration of hydrophilic drug such as GS through the lipophilic stratum corneum layer.
Regarding to the measurements of the flux using a Franz diffusion cell for the different systems, we suggest that the observed decrease in the flux after using DMSO in the ME was because of reducing the solubility of GS in water. However, we observed that GS recrystallized after increasing the DMSO fraction in the ME. Moreover, the decrease in GS concentration when hydrophilic phase increased from 0.5 to 1 ml of GS because of increasing the consumed surfactant amount for preparing clear MEs containing 600 mg from 0.1 to 0.7 ml, led to decrease GS flux in ME-m600mgs+t in comparison to ME- s600mgs+t . Furthermore, the increasing of GS content from 600 mg to 1000 mg in ME b1000mgs+t could led to an increased flux to 1.892 mg/cm²*h. However, the further increasing in encapsulated GS amount to 1200 mg in case system d1200 reduced the flux of GS in comparison to system b1000. That might be due to lesser used surfactant amount or might be related decreasing GS solubility in system d1200 comparing to system b1000. The in vivo bioavailability results showed that using ME as a carrier for GS increased the oral absorption of GS compared to the aqueous solution of GS.

Table 4: Composition of formulated microemulsions with and without gentamicin sulfate

System	Lipophilic phase (IPM) (ml)	Hydrophilic phase (ml)	GS (mg)	Surfactant (ml)	GS (mg/ml)
s600mgs+t	3	0.5 Water	600	0.1 S:T (2:3)	142.86
s600mgs+t Without GS	3	0.5 Water	-----	1.2 S:T (2:3)	
m600mgs+t	3	1 Water	600	0.7 S:T (2:3)	113.2
m600mgs+t Without GS	3	1 Water	--	3.03 S:T (2:3)	
n600mgs+t	3	1 Water:DMSO (0.77:0.233)	600	0.9 Span 20	109.09
n600mgs+t without GS	3	1 Water:DMSO (0.77:0.23)	---	2.06 Span 20	
B1000mgs+t	3	1 Water	1000	0.4 S:T (2:3)	185.19
D1200mgs+t	3	1 Water	1200	0.05 S:T (2:3)	230.5

Using nonionic surfactants, it was possible to formulate stable microemulsions (MEs) containing gentamicin sulfate (GS). These MEs had colloidal characteristics regarding their droplet size and transparency and rheological characteristics. Fourier Transform Infrared Spectroscopy (FTIR) measurements could give evidence of encapsulation of GS inside MEs as well as the orientation of the surfactants around the hydrophilic droplets.

Using MEs as carriers facilitated the penetration of GS through rat's skin as well as via the gastrointestinal tract. Moreover, the flux of GS decreased after decreasing in the solubility through using of DMSO. Furthermore, the increase in the GS concentration increased the flux for a limit then the flux decreased with GS increase. MEs could increase the bioavailability of GS comparing to an aqueons solution but GS still had slow absorption rates in both systems with delayed maximum concentration.

4. Experimental

4.1. Chemicals

Gentamicin sulfate was donated by Dar Al-Dawa pharmaceutical company which was purchased by Dar Al-dawa from Liaoning pharmaceutical Foreign (China). Methanol HPLC grade was purchased from Fulltime (China). Water for HPLC was purchased from LabChem (USA). Dimethyl sulfoxide (DMSO) was purchased from AZ Chem, (Canada). Sorbitan monolaurate (Span® 20) and Poloxyethylenesorbitan.mono-oleate (Tween® 80) were purchased from SIGMA (France). Acetic acid and Isopropyl Myristate (IPM) were purchased from Merck (Germany).

4.2. Microemulsions (MEs) preparation

Gentamicin was dissolved in hydrophilic phase then 3 ml of IPM was added to the solution. The surfactant or surfactant mixture was added dropwise with continuous stirring over a magnetic stirrer to the mixture of lipophilic and hydrophilic phases until a transparent microemulsion was formed. The consumed surfactant amounts were recorded. The different constituents of developed microemulsions are listed in Table 4.

4.3. Pseudo-ternary phase diagrams of microemulsion systems

Three phase diagrams were drawn for the three components: lipophilic phase, surfactant and hydrophilic phase either with 100 mg GS or without GS to find MEs existence area and testing the influence of GS on these areas. In first two three phase diagrams, a mixture of Span 20:Tween 80 (2:3) as surfactant and cosurfactant were used and water as hydrophilic phase. The second two three phase diagrams were stabilized using Span 20 and a mixture of water:DMSO in a ratio of 0.23:0.77 as hydrophilic phase. IPM was used in both of them as lipophilic phase.

Each one of the three components, surfactants, hydrophilic phase and lipophilic phase forms a face of the triangles. Two ml of a formulation were made according to the faction of the three components in each cross point of the three parallel lines to the three faces of the triangle. More formulations were made between the cross points on the border of MEs area. Only the clear, stable formulation after mixing added to black ME existing area.

4.4. Viscosity measurement

An electric Rheometer made by Anton pear, universal tool, model MCR 301 (Germany) was used to determine the viscosity and rheological properties of MEs. Rheograms were established for the MEs with increasing and decreasing shear force at 25 °C on the bob and cup viscometers.

4.5. Droplet size measurement (Zeta-potential measurement)

A laser Doppler electrophoresis was carried out on the microemulsions with GS and without GS using a Zeta-sizer made by Microtrac (USA) which is capable of measuring Particle size ranging between 0.8 nm to 6.54 mm, Zeta potential Range-125 to +125 mV.

4.6. Fourier Transform Infrared Spectroscopy (FTIR) measurements

The encapsulation of GS as well as the structure of the MEs were evaluated by recording of spectra using FTIR spectrometer (Perkin Elmer UATR Two, Li600301 spectrum (UK)) for each single component, ME with and without GS. The FTIR-spectra were measured between 450 and 3950 cm^{-1}.

4.7. Preparing rat's skin

Wistar rats (220 – 250 g, 10 –12 weeks old) were supplied originally from the University of Jordan and fertilized at the animal house of Isra University. All procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals which were approved by the Animal Ethics Committee of Isra University. The rats were shaved carefully before executing. Then skin was peeled. The peeled skin was cleaned from adipose tissues carefully. The skin was cut to small pieces to fit with Franz diffusion cell surface area (the diameter is a bit larger than 15 mm) then washed with buffer and stored in a deep freezer at a temperature of -70 °C.

4.8. Study of GS penetration using Franz diffusion cell

A single Franz diffusion cell which was used for studying the penetration of GS through rat's skin made by Hanson in USA (15 mm diameter, 7 ml acceptor volume) fitted with a thermo circulator water bath to adjust the temperature of the cell at 32±1 °C. A frozen rat skin piece was removed from the freezer and thawed in a water bathe at 32 °C immediately before using in the Franz-diffusion cell. The skin was fixed with a ring over the acceptor compartment medium. The skin piece was tested against the light for its integrity. The acceptor compartment was filled with 7 ml of water. Only 0.1 ml of each microemulsion was applied over the skin. A flange was used to fix a glass disc and the ring over the donor compartment with acceptor compartment. 0.5 ml were removed after 1, 2, 3,4,5,6, 7 and 24 h for analyzing the penetrated drug through the skin using HPLC. The removed sample was replaced by equal volume of the same acceptor medium.

4.9. Study of GS bioavailability in rats

A 0.5 ml either of ME-b1000 or a 100 mg/ml GS solution in water was applied orally to four white male rats for each preparation. A 0.5 ml blood sample was collected after 0.5, 1, 2.5, 4. 5, 6, 9, 13, 16, 20, 25 h in EDTA-blood-tubes. The blood samples were centrifuged at 4000 rpm at 4 °C for 20 min. The plasma parts were transferred into new tubes then 0.1 ml of acetonitrile was added into each tube. The tubes were centrifuged at rate of 5000 rpm at 4 °C for 15 min. The supernatant were transferred to HPLC sample tube and diluted till 0.5 ml with water.

4.10. Gentamicin sulfate (GS) analyzing using high pressure chromatography (HPLC) method

Quantification of GS was performed on Thermo scientific, Dionex Ultimate 3000 HPLC chromatographic (Germany) connected with diode array detector using suitable standards. 20 µl were injected into the column system C18 4.6*250 mm and separated using a mobile phase of water:methanol in a ratio of 70:30 at a flow rate of 1 ml/min and GS was detected at wave length of 280 nm for assaying GS in removed samples from Franz diffusion cell. Acetic acid (4 ml) was added to each 1 l of a mixture of water and methanol in a ratio of 3:7 for detection of GS in purified blood samples at a wave length of 214 nm and at a flow rate of 1.5 ml/min.

4.11. Pharmacokinetic and statistical analysis

All the tests either the analysis or penetration studies are triplicated. The mean value and standard deviation are calculated. Origen program was used for the statistical evaluation with a confidence interval of 95%. The steady state flux (Jss) was calculated from the slope at steady state line (Eq.1) by plotting the penetrated amount per cm^2 (Q/A) against the time (t) as in Eq. 1 and 2 (Li et al. 2003):

$$Q/A = J_{ss} * t \quad (1)$$

From Eq. 1:

$$J_{ss} = \frac{Q}{A(t - t_{lag})} = K_p C_v \quad (2)$$

From Eq. 2 we can find the permeability coefficient (K_p):

$$K_p = \frac{K_{sc} D_{sc}}{h_{sc}} = \frac{J_{ss}}{C_v} \quad (3)$$

D_{sc}: diffusion coefficient through stratum corneum
K_{sc}: partition coefficient between the excipient and the SC,
A: skin surface area
Q: the cumulative mass penetrating a membrane
C_s: the constant drug concentration in the donor solution,
And h_{sc}: the thickness of the membrane or the diffusion path length or stratum corneum.
The absorption rate constant, elimination rate constant and area under the curve were estimated from plasma level against time data after oral application of preparations using pheonix® program (Phoenix Version 7.0, Certara, L.P.) by applying one compartment open model. Moreover the percentage relative bioavailability (PRBA) was calculated using eq.4.

$$PRBA = \frac{[AUC]_{ME} / Dose_{ME}}{[AUC]_{solution} / Dose_{solution}} \times 100 \quad (4)$$

Conflicts of interest: None declared.

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