PREPARATION AND CHARACTERIZATION OF NIOSOMES CONTAINING METRONIDAZOLE

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Abstract

In the present study the preparation and the characterization of non-ionic surfactant vesicles (NSV, niosomes) containing metronidazole are described. NSV were prepared by lipid film hydration technique using non-ionic surfactants with cholesterol at 1:2 and 1:1 ratio. At the same time NSV were characterized by melting points, by IR spectroscopy, by TG/DTG (thermogravimetry/derived thermogravimetry) and entrapment efficiency of metronidazole (metronidazole percent concentration). The maximum metronidazole entrapment was observed in the case of non-ionic surfactant/cholesterol use at 1:1 ratio.

Rezumat

Acest studiu prezintă prepararea şi caracterizarea veziculelor non-fosfolipidice (NSV, niozomi) ce conţin metronidazol. Niozomii au fost preparaţi prin tehnica hidratării filmelor lipidice utilizând tensioactivi neionici şi colesterol în proporţie de 1:2 şi 1:1. Niozomii (NSV) au fost caracterizaţi prin determinarea punctelor de topire, realizarea spectrelor IR, realizarea termogramelor TG/DTG şi studiul eficienţei de încorporare a metronidazolului. Cantitatea maximă de metronidazol încorporată s-a observat la niozomii preparaţi cu tensioactivi neionici/colesterol (1:1).

Keywords: metronidazole, niosomes, entrapment, surfactants

Introduction

Metronidazole is an antibacterial, antiprotozoal and amoebicide substance used in systemic treatment of anaerobic bacteria and protozoa

infections and in topical treatment of dermatological conditions, such as rosacea [8, 9, 13].

For an optimal drug action, drugs molecules could be transported by a carrier to the site of action and released in order to achieve action.

Niosomes (non-ionic surfactant vesicles, NSV) are vesicle systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs [2, 3]. Niosomes are microscopic lamellar structures formed on a mixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media [7, 12]. These vesicles appear to be similar in terms of the physical properties to liposomes, being prepared in the same way, but are characterized by a higher chemical stability with respect to liposomes due to the difference between surfactants and phospholipids.

The chemical stability as well as the relative low cost of the materials used to prepare niosomes made these vesicles more attractive than liposomes for the industrial production, both in pharmaceutical and cosmetic applications.

Niosomes as delivery devices have also been studied with anticancer, antitubercular, anti-leishmanial, anti-inflamatory, hormonal drugs and oral vaccines [1, 6, 11, 14, 16]. These vesicles have been reported to decrease side effects, give sustain release and to enhance penetration of the trapped substances through skin. The reduction of the barrier properties of *stratum corneum* resulting from the property of vesicles as a penetration enhancer, are realized both by reducing trans-epidermal water loss and by increasing smoothness *via* replenishing lost skin lipids [10]. Many drugs such as estradiol, tretinoin, dithranol, enoxacin, non-steroidal anti-inflammatory drugs, imidazoles have been successfully encapsulated in niosomes for topical application. The entrapment efficiency of the vesicles depended on alkyl chain length of non-ionic surfactants and amount of cholesterol used to prepare vesicles [4, 5, 17]. We aimed to obtain niosomes with metronidazole, because targeted delivery of metronidazole *via* niosomes can increase efficiency of the drug in topical treatment.

Materials and Methods

Reagents

Span 40 ($C_{22}H_{42}O_6$, polyoxyethylene sorbitan monopalmitate), Brij 58 ($C_{16}H_{33}(OCH_2CH_2)_nOH$, polyoxyethylene monocetyl ether), Myrj 52 ($C_{17}H_{35}COO(CH_2CH_2O)_nH$, polyoxyethylene monostearate) were purchased from Serva-Heidelberg (Germany). Metronidazole and cholesterol were purchased from Merck Co. (Germany). All other chemicals and solvents

were of analytical purity. All solutions were prepared with bidistiled water. Phosphate buffer saline (PBS) was prepared as described in the Xth Romanian Pharmacopoeia [19].

Vesicles preparation

Niosomes were prepared using the lipid film hydration technique [3, 4, 15, 18]. Metronidazole non-ionic surfactant and cholesterol were weighed as indicated in table I and dissolved in chloroform/methanol system (1:2) in a 250 mL round bottom flask. Two types of niosomes were prepared: non-ionic surfactant vesicles I (NSV I) with 1:2:2 ratio of those three components (metronidazole:non-ionic surfactant:cholesterol) and non-ionic surfactant vesicles II (NSV II) with 1:1:1 ratio of the same components.

Table I

Composition of the niosomes

Components	HLB	NSV I			NSV II		
		P_1	P_2	P_3	P'_1	P'_2	P'_3
Metronidazole	-	1	1	1	1	1	1
Brij 58	15.8	2	-	-	1	-	-
Myrj 52	16.9	-	2	-	-	1	-
Span 40	6.7	-	-	2	-	-	1
Cholesterol	1.0	2	2	2	1	1	1

HLB – hydrophilic-lipophilic balance
NSV – non-ionic surfactant vesicles

The solvent mixture was evaporated in a rotary evaporator, under vacuum, at 27 ± 2^{0}C and 70-80 rpm flask rotation, until a dry, white lipid film was obtained.

The obtained film was hydrated with 30 mL of PBS with pH = 7.4 for 4 hours, at 27 ± 2^{0}C and 20 rpm flask rotation, until a white suspension was obtained. The niosomal suspension was further hydrated at 4^{0}C, in the absence of light, for 24 hours. The niosomes were then separated by centrifugation at 2500 rpm for 30 minutes, followed by drying in a vacuum exiccator at 25^{0}C. The outputs were calculated.

Characterization of niosomes

The prepared niosomes were analyzed for melting points with a Melt-Temp R apparatus equipped with a digital thermometer. The IR spectra were performed with DIGILAB Scimitar Series spectrophotometer, using KBr pellet technique. The TG/DTG curves have been recorded with Mettler Toledo TGA-SDTA851^e derivatograph. The registrations were performed under nitrogen, with a rate of 20 mL/min., over a temperature ranging from 25^{0}C to 900^{0}C and at a heating rate of 10^{0}C/min. The samples weight was 3-

5 mg and the operational parameters were constant for all samples in order to obtain comparable data. The processing of curves was achieved with STAR software. Prepared niosomes were analyzed for percent drug entrapment by a spectrophotometric method [19], after the separation of free drug. We used a Jasco UV-VIS V-530 spectrophotometer and the detection was performed at λ = 277 nm, using a 0.001% standard solution of metronidazole in 0.1 M HCl. Three weighings were made for each of the six niosomes variants, for which the absorbance was determined. Using external standard method we determined the percent of metronidazole entrapment.

Results and Discussion

The melting points of the obtained NSV are listed in table II.

Table II

The melting points of the metronidazole NSV

Substances	**Melting points (^{0}C)**
Metronidazole (Merck)	158-162
Brij 58 (Serva)	39-45
Myrj 52 (Serva)	38-43
Span 40 (Serva)	42-46
Cholesterol (Merck)	147-150
P_1	135-137
P_2	129-132
P_3	114-118
P'_1	133-136
P'_2	153-155
P'_3	160-162

The NSV obtaining outputs are listed in table III.

Table III

The NSV obtaining outputs

Sample	P_1	P_2	P_3	P'_1	P'_2	P'_3
Output (%)	76.80	70.40	71.70	83.20	76.45	78.52

The IR spectra of metronidazole revealed a band in the frequency range of 1369.46 cm^{-1} and 1535.33 cm^{-1}, corresponding to nitro group vibration.

The IR spectra of the obtained NSV display the characteristic vibrations of the nitro group in the niosomes molecule: for P_1 niosomes the intense sharp band at 1350.17 cm^{-1} and 1377.1 cm^{-1}; for P_2 niosomes the intense sharp band at 1350.17 cm^{-1} and 1465.9 cm^{-1}; for P_3 niosomes the intense sharp band at 1377.17 cm^{-1} and 1564.27 cm^{-1}; for P'_1 niosomes the

intense sharp band at 1371.3 cm^{-1} and 1535.33 cm^{-1}; for P'_2 niosomes the intense sharp band at 1369.46 cm^{-1} and 1537.26 cm^{-1}; for P'_3 niosomes the intense sharp band at 1371.39 cm^{-1} and 1537.26 cm^{-1}. The IR spectra of all studied NSV revealed the presence of the characteristic band for nitro group, which demonstrated that metronidazole was entrapped in the vesicles.

From the TG/DTG curves (figure 1, figure 2, table IV and table V) it can be remarked specific temperatures of decomposition for each prepared NSV. The thermic degradation of all samples is realized in I to IV steps. The prepared NSV have a particular thermal behavior compared with parent molecules. They exhibit an increased thermal stability evidenced by higher decomposition temperatures than those of metronidazole.

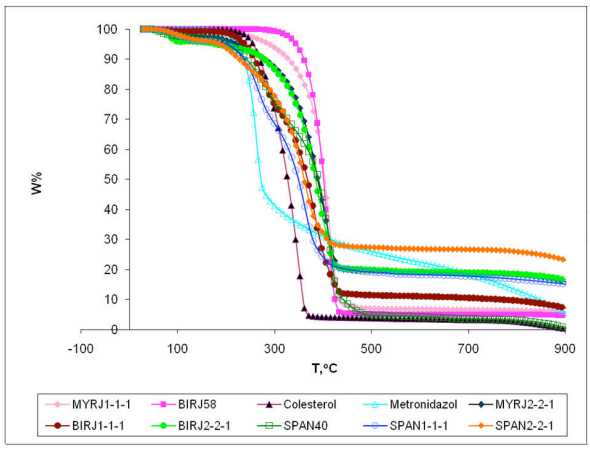

Figure 1
Thermogravimetric curves - TG curves

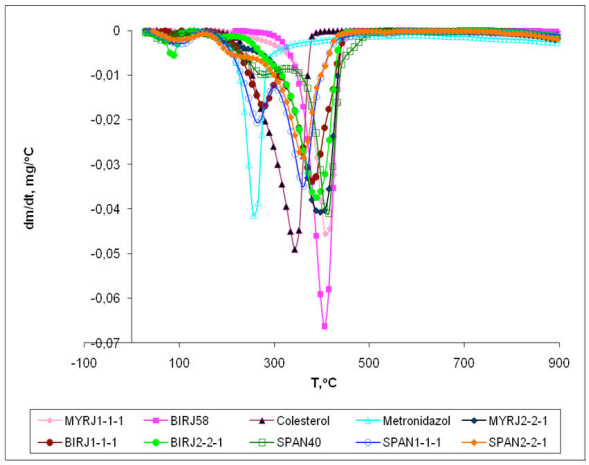

Figure 2
Thermogravimetric curves - DTG curves

Table IV

Thermogravimetric characteristics of parent substances

Compound	Step	T_{onset} (^{0}C)	T_{peak} (^{0}C)	T_{endset} (^{0}C)	Weight loss (%)	Residue (at 900^{0}C)
Metronidazole	I	218	260	277	73.26	0
	II	609	-	900	23.66	
Brij 58	I	349	407	428	96.04	3.96
Span 40	I	52	63	108	2.97	0
	II	212	275	375	40.79	
	III	375	412	478	56.24	
Cholesterol	I	246	346	364	100	0

Table V

Thermogravimetric characteristics of the prepared NSV

Compound	Step	T_{onset} (^{0}C)	T_{peak} (^{0}C)	T_{endset} (^{0}C)	Weight loss (%)	Residue (at 900^{0}C)
P_1	I	76	85	93	4.04	15.89
	II	256	386	423	80.07	
P_2	I	76	81	104	2.22	15.38
	II	326	401	428	82.40	
P_3	I	75	86	134	3.83	22.04
	II	198	360	416	74.13	
P'_1	I	229	279	298	28.31	6.99
	II	337	382	429	64.70	
P'_2	I	226	271	291	29.68	4.36
	II	335	373	429	65.96	
P'_3	I	76	103	124	3.94	14.33
	II	222	265	283	29.91	
	III	336	364	420	46.83	
	IV	420	539	900	4.99	

The temperature corresponding to the maximum mass lost rate increased from 260^{0}C for metronidazole to values between 360 to 539^{0}C for the prepared NSV. If we consider the T_{onset} as a stability criterion, we can obtain a series of thermostability: $P_3 < P'_3 < P'_2 < P'_1 < P_1 < P_2$.

Figure 3 shows the percent of metronidazole entrappement of the prepared vesicles. In table V are presented the percent of metronidazole entrapped average of the three determinations for each prepared NSV, the standard deviation (SD) and the relative standard deviation (RSD).

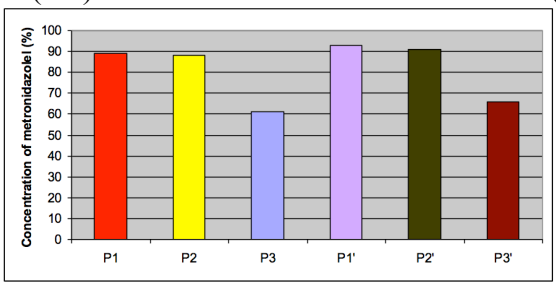

Figure 3

Percent of metronidazole entrappement in the NSV

Table V

Percent of metronidazole entrappement in the NSV

Type of niosome	Percent of metronidazole entrappement (%) ± SD	RSD
P_1	89.03 ± 0.0416	0.0467
P_2	88.32 ± 0.02	0.0226
P_3	61.32 ± 0.02	0.0326
P'_1	92.73 ± 0.0152	0.0164
P'_2	91.22 ± 0.02	0.0219
P'_3	65.81 ± 0.02	0.0303

All characteristics of NSV show a great metronidazole entrapment of vesicles prepared with 1:1:1 ratio of metronidazole:non-ionic surfactant:cholesterol compared to the vesicles prepared with 1:2:2 ratio of metronidazole:non-ionic surfactant:cholesterol.

The metronidazole entrapment decreases as follows: $P'_1 > P'_2 > P_1 > P_2 > P'_3 > P_3$. Within each niosomes range, the decrease in entrapment is as follows: Brij 58 > Myrj 52 > Span 40.

Surfactants with longer alkyl chains generally give larger vesicles. However, the surfactants with high hydrophilic properties and high HLB, as Brij 58, are preferable to form micelle in aqueous solutions. Surfactants with lipophilic properties, as Span 40, did not improve the solubility and the metronidazole entrapment was lower.

There were calculated the niosomes amount corresponding to one gram of metronidazole and the obtained results are listed in table VI.

Table VI

The niosomes amounts corresponding to one gram of metronidazole

Sample	P_1	P_2	P_3	P'_1	P'_2	P'_3
Niosomes amounts (g)	1.1238	1.131	1.630	1.027	1.098	1.518

NSV obtained from the cetyl chain (C_{16}/surfactants) in the presence of cholesterol at 1:1 ratio, were bilayer membranes less rigid and more deformable than those obtained from stearyl chain (C_{17}/surfactants).

Conclusions

Bilayer vesicles can be prepared with the mixtures of some non-ionic surfactants and cholesterol in order to efficiently entrap metronidazole.

The IR spectra of all studied NSV revealed that metronidazole was entrapped in the vesicles. At 1:1 ratio of surfactants and cholesterol these vesicles were thermaly stable compared to metronidazole, able to efficiently entrap metronidazole and the membrane was more deformable and yet stable. A higher metronidazole entrapment was observed with non-ionic

surfactant/cholesterol at 1:1 ratio, and the maximum metronidazole entrapment was observed with Brij 58, as non-ionic surfactant.

The role of these metronidazole NSV, obtained in this study, can only be settled following future *in vitro* and *in vivo* investigations.

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Manuscript received: September 4th 2011