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SOLUBILIZING PROPERTIES OF NEW SURFACE-ACTIVE AGENTS, PRODUCTS OF CATALYTIC OXYETHYLATION OF CHOLIC ACID

MICHAŁ KRZYSZTOF KOŁODZIEJCZYK[1]*, MICHAŁ JAKUB NACHAJSKI[1], MAREK LUKOSEK[2] and MARIAN MIKOŁAJ ZGODA[1]

[1]Chair of Applied Pharmacy, Medical University of Lodz, ul. Muszyńskiego 1, 90-151 Łódź, Poland
[2]Surface-Active Agent Plant "ICSO Blachownia", ul. Energetyków 9, 47-225 Kędzierzyn-Koźle, Poland

Abstract: Solubilizing properties of aqueous solutions of a series of surface-active agents, products of oxyethylation of cholic acid, were examined in the present study. The content of oxyethylated segments determined by means of the ^{1}H NMR method enabled the verification of the molecular mass of surfactants along with the calculation of the structural hydrophilic-lipophilic balance (HLB), the solubility parameter $\delta^{1/2}$, and the required solubility level of balance HLB_R. Viscosimetric measurements enabled the calculation of the limiting viscosity number, the content-average molecular mass, the effective volume, the hydrodynamic radius of the surfactant micelle and their equilibrium adducts with rutin, diclofenac and loratadine (BCS Class II and III). By means of the spectrophotometric method (UV) the amount of the solubilized diclofenac, loratadine and rutin (rutoside) was determined in the equilibrium system (saturated solution) in the environment of aqueous solutions of cholic acid derivatives of n_{TE} = 20-70. The obtained results serve as a basis for determining the solubilization mechanism of lipophilic therapeutic products and indirectly for estimating the influence of the above process on pharmaceutical as well as biological availability of a micellar adduct from model drug forms (Lindbladt lithogenolitic index).

Keywords: oxyethylation, cholic acid, aqueous solutions, solubilization, hydrophilic-lipophilic balance, hydrodynamic values, HPLC analysis, Tweens

The aim of this preformulation research was to use a selective catalyst to create new surface-active agents, products of oxyethylation of cholic acid, with various content of n_{TE} = 70 (n_{TE} = number of oxyethylene segments).

Estimation of selected hydrodynamic values, surface activity (γ), and the level of HLB (hydrophilic-lipophilic balance) may serve as the basis for estimating the application characteristics of homologous derivatives. Moreover, it may be used for further preformulation research on their solubilizing properties for selected therapeutic agents belonging to BCS (Biopharmaceutical Classification System) Class II and III (1-17).

This aspect of research on the catalytic process of cholic acid oxyethylation was integrated with chromatographic (HPLC, high-performance liquid chromatography and GPC, gel permeation chromatography) comparative analyses of molecular mass dispersion in obtained product, and particularly its content of polyethylene glycols (PEG).

The Tween type polysorbates (Tween-40, -60, -61, -80, and -85) were considered as the reference class of surfactants. Currently, these compounds are widely used in drug and cosmetic form technology, where they serve as hydrophilizers of pill mass, emulsifiers and micellar solubilizers for lipophilic therapeutic agents (18-20).

The results obtained facilitate innovative preformulation research on the creation of solid oral dosage form with a physiologically stable process of pharmaceutical availability and the process of mass exchange at phase boundary. We expect that the synthesized class of non-ionic surfactants will be recognized as xenobiotics by the human enzymatic systems, and will markedly modulate the physiological value of lithogenolithic index of bile A (so-called Lindblat index) (21).

EXPERIMENTAL

Reagents

Oxirane (ethylene oxide, Mazowsze Refinery and Petrochemical Plant "Petrochemia", Płock, Poland); NaOH catalyst employed in the process of oxyethylation (Surface-Active Agent Plant "ICSO

* Corresponding author: e-mail: michal.kolodziejczyk@umed.lodz.pl

Blachownia", Kędzierzyn-Koźle, Poland); cholic acid, $C_{24}H_{40}O_5$, ROTH (Carl Roth GmbH, Germany); diclofenac, 2-[2-[(2,6-dichlorophenyl) amino]phenyl]acetic acid (Sigma, Germany); rutin (Rutosidum), analytically pure (Sichuan Xieli Pharmaceutical Co. Ltd., China); loratadine, analytically pure (Zydus Cadila – Cadila Healtcare Ltd., India); polysorbates, polyoxyethylene sorbitan fatty acid esters (Tweens): Tween-40 (Aldrich), Tween-40 (Fluka), Tween-60 (Loba), Tween-60 (Serva), Tween-61 (Koch-Light), Tween-80 (Suchardt), Tween-80 (POCH Gliwice, Poland), Tween-85 (Atlas), and Tween-85 (Loba). Basic physicochemical and application characteristics of Tweens as widely used excipients of cosmetics and pharmaceutical products were presented in numerous monographs and publications (22-24).

Synthesis of the oxyethylation products of cholic acid

Oxyethylation of cholic acid was conducted with the use of an oxyalkylation facility located at the Surface-Active Agent Plant Institute of Heavy Organic Synthesis - "ICSO Blachownia" in Kędzierzyn-Koźle. The products of oxyethylation of cholic acid with the declared content of n_{TE} = 70 were obtained with the use of selective catalyst (NaOH) as previously described (8, 16). Chart 1 illustrates the course of the oxyethylation process. The parameters of oxyethylation process are summarized in Table 1. Established weight-average molecular mass of cholic acid (M_w) was 408 g/mol. The synthesis was carried out in an inert solvent – high purity mineral oil (Ondina, Stell). After the completion of synthesis and cooling, the final product (mineral oil phase) was separated by decantation and drying.

HPLC determination of the average molecular mass dispersion in the products of catalytic oxyethylation of cholic acid and in the reference polysorbates

HPLC determination of molecular dispersion in the products of catalytic oxyethylation of cholic acid and in the reference polysorbates (GPC) is based upon separating the sample into molecules of various hydrodynamic volumes. GPC is considered a relative technique due to system calibration and maintenance of stable analytical conditions. The system was calibrated with standard polystyrene structures with linear structure and known physicochemical parameters such as content-average (M_h) and weigh-average (M_w) molecular mass.

Equipment

Chromatographic set: L7100 pump (Merck Hitachi) with degasifier (Knauer) and manually dosing pump (Knauer) with 20 mL loop volume, refractometric detector (Varian) with the "Grams-386 for chromatography" software (Galactics) for collection and analysis of experimental data.

Conditions of chromatographic analysis

Column: connected to pre-column 50 × 4.6 mm, eluent (THF, tetrahydrofuran) flow rate 0.3 cm^3/min. At 30 ± 0.1°C column temperature, the deviation of retention time amounted 0.2%. Retention time of all chromatographs was corrected by adjusting the retention time of systemic peak to constant value 22.350 ± 0.001 min. The system was calibrated with polystyrene (PS) molecular mass standards (Polimer Laboratories); PS with M_w = 98300 (Aldrich) and PS with M_w = 500 (Fluka). The concentration of prepared calibration solutions ranged from 10 to 16 mg PS per 10 cm^3 of eluent.

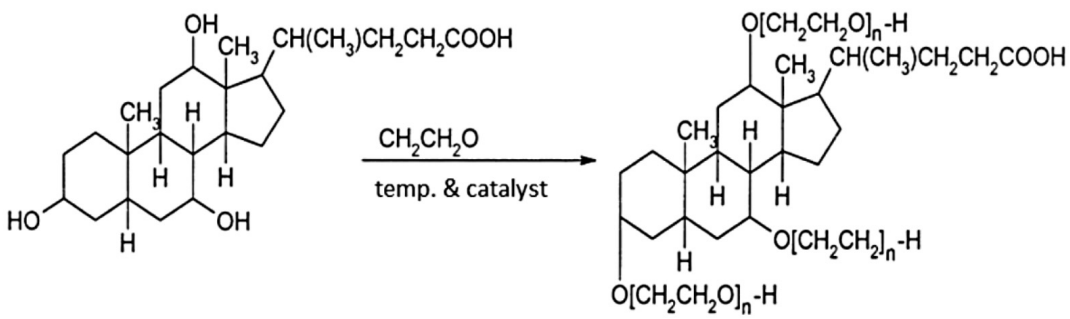

Chart 1. Schematic presentation of the cholic acid oxyethylation process. Selected parameters of the oxyethylation process of cholic acid. Powdered catalyst, NaOH

Table 1. Characteristics of the oxyethylation of cholic acid.

Number of sample	239/08	256/08	284/08	292/08	308/08	319/08	324/08
Substrate (cholic acid, g)	100	100	40	40	40	40	40
Solvent (Ondina oil, g)	200	200	260	260	260	260	260
Mean degree of oxyalkylation	10	20	30	40	50	60	70
Catalyst (NaOH, g)	0.41	0.26	0.17	0.47	0.26	0.30	0.34
Ethylene oxide (g)	108	216	129	172	216	259	362
Temperature of synthesis (°C)	160	160	160	160	160	160	160
Minimal synthesis pressure (Kpa)	200	200	200	200	200	200	200
Maximal synthesis pressure (Kpa)	300	350	300	300	300	300	300
Duration of synthesis (min)	215	251	225	180	200	185	270

Separation efficiency of the column determined for PS 9200 and PS 98300 amounted 4.32.

Determination of the polyethylene glycol (PEG) content in the product of cholic acid oxyethylation and in the reference polysorbates

Total concentrations of PEGs in the product of cholic acid oxyethylation and in the reference polysorbates were determined by means of reversed-phase liquid chromatography with 65% aqueous solution of acetonitrile as eluent. Quantitative determination was performed onto the ODS 250 × 2.1 mm column with ELSD detector (evaporative light scattering detector). The detection is based on spraying and evaporation of the column eluent, and intensity determination of light scattered by the obtained dispersion system (spray). The column was calibrated with the PEG 1000 standard (Fluka) based on the following relationship between the concentration progression from 0.48 to 2.40 mg per 10 mL, and the peak area (mm^2):

$$P(mm^2) = f(c, mg/mL)$$

This aforementioned relationship was described by the following approximation formula (by p = 0.05 and $r^2 \geq 0.9950$):

$$P(mm^2) = 12.5150 \times c(mg/mL) - 6.4748$$

which was further transformed into the following application version:

$$c = P + \frac{6.4748}{12.5150}$$

and enabled the estimation of PEG contents in the oxyethylation product of cholic acids and in reference polysorbates.

Example chromatograms of the cholic acid oxyethylation products and reference polysorbates are presented in Figures 1 and 2, respectively. The results of chromatographic analysis characterizing the homologous lines of the cholic acid oxyethylation products and reference polysorbates are summarized in Tables 2 and 3.

Estimation of the number of oxyethylated segments (n_{TE}) and $HLB_{^1H\ NMR}$

The ^1H NMR spectra of the products of oxyethylation of cholic acid were obtained as described previously (8, 16). They were used to calculate the hydrophilic-lipophilic balance on the basis of the following equation:

$$HLB_{^1HNMR} = \frac{15 \times A_h}{0.05(15 \times A_h + 10 \times A_l}$$

where: A_h – number of hydrophilic protons; A_l – number of lipophilic protons.

Table 2. Determined by means of gel chromatography, content-average (M_h) and weight-average (M_w) molecular mass, M_w/M_h, ratio, and retention time of the peak maximum (t_p) of the products of cholic acid oxyethylation.

Product of cholic acid oxyethylation Sample number	Peak M_p	Content (%)	M_η	M_w	M_w/M_η	t_p	M_p	PEG content (%)
Cholic acid	695 305 138 92	96.57 0.75 1.47 1.21	596	667	1.120	18.17	695	-
Cholic acid x n_{TE} = 10 239·08·BD	3255 761 320 139 94	69.27 28.59 1.37 0.27 0.50	1315	3425	2.60	18.04	761	31.71
Cholic acid x n_{TE} = 20 256·08·BD	3715 1343 301 138 102	64.73 33.07 1.05 0.88 0.27	1772	4500	2.54	17.16	1343	17.86
Cholic acid x n_{TE} = 30 284·08·BD	3639 1978 733 313 117	53.71 35.74 9.07 1.03 0.45	1943	5002	2.57	16.47	1978	22.66
Cholic acid x n_{TE} = 40 292·08·BD	4215 2408 715 296 103	43.79 40.61 9.89 2.94 2.77	1195	4644	3.88	16.09	2408	27.13
Cholic acid x n_{TE} = 50 292·08·BD	7601 4279 743 315 120 91	17.60 31.54 45.61 3.90 1.16 0.19	957	3961	4.14	18.78	743	10.62
Cholic acid x n_{TE} = 60 319·08·BD	5258 3019 658 285 115	48.26 45.12 2.96 2.19 1.47	1885	6519	3.46	15.64	3019	40.96
Cholic acid x n_{TE} = 70 324·08·BD	7787 4143 739 313 137 95	51.41 45.73 1.42 0.36 0.13 0.95	3047	11349	3.72	15.03	4143	42.52

Determination of the overall number of lipophilic protons $\Sigma^{l}H = 36$ in the structure of the molecule of cholic acid made it possible to calculate the content of oxyethylated segments in the product on the basis of the following equation (8, 16):

$$n_{TE} = (36 \times \frac{A_h}{A_l} - 3)/4$$

The estimated content of $n_{TE(x)}$ enabled calculation of the weight-average molecular mass (M_w) on the basis of the equation:

$$Mw = 408.58 + n_{TE(x)} \times 44.053$$

Determination of drop-point temperature (T_K)

Drop-point temperature was determined in accordance with the Polish Pharmacopoeia (FP VIII) and respective Polish Standard (16). The obtained values of T_K were used to calculate the predicted viscosity of the surfactant at the drop-point on the basis of the following equation:

$$\eta = 5.1 \times 10^{-4} \times M^{1/2}_{w} \times T^{1/2}_{K} \times V^{-2/3}_{S}$$

which was further transformed into the following application version:

$$\eta = 5.1 \times 10^{-4} \times \sqrt{M_w} \times \sqrt{T_K} \times \frac{1}{\sqrt[3]{V^2_S}}$$

where: T_K – drop-point temperature (273 + t°C), M_w – weight-average molecular mass (g/mol), V_S – molar volume ($cm^3 \times mol^{-2}$) calculated by means of the Fedors method (18). Drop-point temperature was employed to estimate the volume expansion coefficient (α_{Vo}) and the limited expansion coeffi-

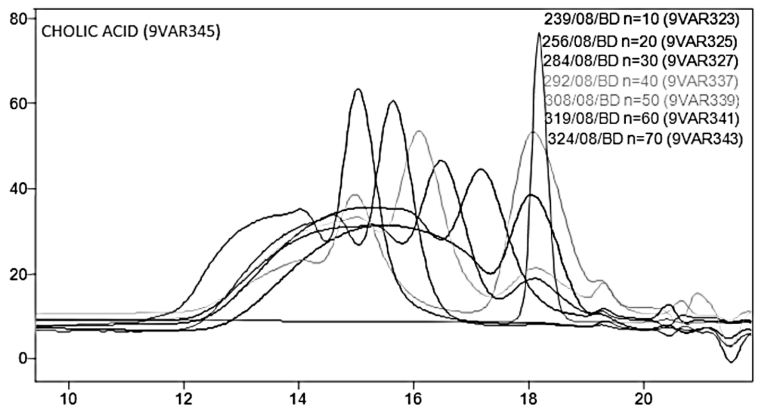

Figure 1. Example chromatograms of the cholic acid oxyethylation products

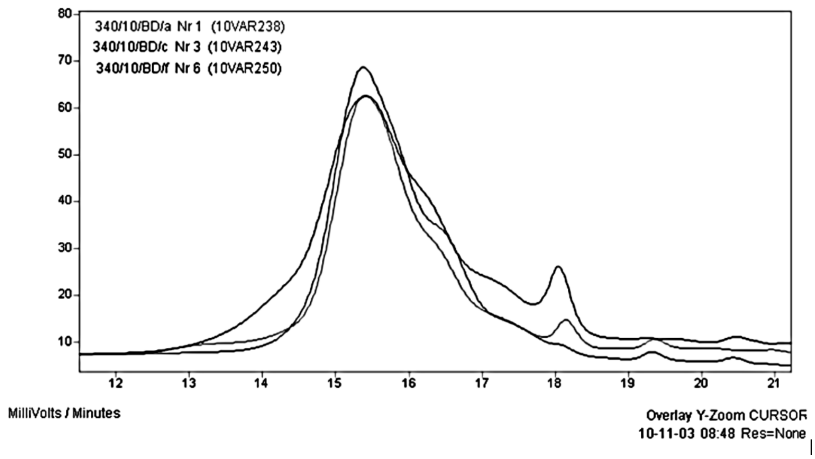

Figure 2. Example chromatograms of reference polysorbates

Table 3. Determined by means of gel chromatography, content-average (M_h) and weight-average (M_w) molecular masses, M_w/M_h ratio, retention time of the peak maximum (t_p), and the estimated PEG content (%) in the Tween type polysorbates (Tween-40, -60, -61, -80, and -85).

Polysorbate Polysorbate	Peak M_p	Content (%)	M_η	M_w	M_w/M_η	t_p	M_p	PEG content (%)
Tween 40 (Fluka)	2777 1052 682 295 132	995	2359	2.37	15.81	2777	75.44 8.64 5.51 6.62 3.79	31.84
Tween 40 (Aldrich)	2871 1126 686 244 137	824	2202	2.67	15.74	2871	67.64 6.97 12.69 7.08 5.62	28.01
Tweed 60 (Loba)	3268 1825 778 269 137	715	2472	3.45	15.48	3268	52.75 17.34 14.42 7.95 7.54	22.33
Tween 60 (Serva)	2487 499 239 134	1037	2400	2.31	13.48	2487	89.54 3.42 3.91 3.13	44.22
Tween 61 (Koch-Light)	2585 2058 1424 780 296 147	772	1547	2.00	16.39	20.58	17.89 22.09 27.81 18.08 10.66 3.47	28.66
Tween 80 (Schuchardt)	3119 794 267 153	933	3523	3.77	15.57	3119	78.00 10.98 6.35 4.67	30.95
Tween 80 (Difica)	13847 3403 717 287 106	875	4307	4.92	15.40	3403	7.94 72.04 6.98 10.11 2.39	26.11

Table 3. cont.

Polysorbate Polysorbate	Peak M_p	Content (%)	M_η	M_w	M_w/M_η	t_p	M_p	PEG content (%)
Tween 80 (POCH-Gliwice)	3448 1185 301 139	1012	2912	2.88	13.39	3448	61.55 27.24 7.16 4.05	26.54
Tween 85 (Loba)	2893 2156 1492 713 297 135	1272	3286	2.58	12.36	713	41.19 19.13 17.39 17.79 3.64 0.85	17.16
Tween 85 (Atlas)	3301 2101 1496 711 285 128	888	6068	6.83	16.35	2101	45.02 18.84 14.92 9.90 5.95 5.37	7.44

cient (α_{lo}) of the surfactant (polymer) during an equilibrium change of physical state by means of the Lennard-Jones equation:

$$T_k = \frac{\alpha}{4\beta_l} \times \frac{1}{\alpha_{Vo}}$$

after the introduction of the constant:

$$\frac{\alpha}{4\beta_l} \times \frac{1}{42}$$

where: α and β - constant coefficients of Lennard-Jones equation and an application transformation:

$$\alpha_{Vo} = \frac{1}{42} \times \frac{1}{T_k}$$

The calculated values characterizing the structure of the products of oxyethylation of cholic acid are presented in Table 4.

Viscosity and surface activity of aqueous solutions of the products of cholic acid oxyethylation

The limiting viscosity number LVN [η] of aqueous solutions of the products of oxyethylation of cholic acid was estimated on the basis of the Polish Standard by means of the Ubbelohde viscosimeter (19). The estimated limiting viscosity number was used to calculate several viscosity values: M_η – viscosity average molar masses, R_o – end of mean square distance between chain terminals, R_{obs} – hydrodynamic value of micelle radius, Ω – effective volume, and the solubilization index $n_{/s/}$. The results are summarized in Table 6.

Micellar solubilization of lipophilic therapeutic agents in the environment of aqueous solutions of cholic acid oxyethylation

Previously described spectroscopic method (12-15) was employed to determine the amount of BCS Class II and III lipophilic therapeutic agents solubilized in equilibrium conditions in aqueous solutions of novel surface-active agents with $c_{exp} \geq$ cmc (exposure concentration ≥ critical micelle concentration) (17). The transformation of regression equations with p = 0.05 to the form:

$$c_{/s/} = A - \frac{a}{b}$$

where A – absorbance; a, b – coefficients of equation makes it possible to calculate the amount of the solubilized therapeutic agent. The results of the calculation can be used to estimate the value of the micellar partition coefficient.

Table 4. Drop-point temperature (T_K, t_k°C + 273.1) and selected physicochemical characteristics of liquefied products of the cholic acid oxyethylation.

Product of cholic acid oxyethylation · n_{TE}	ΣΛE; cal/mol[1]	ΣΛV; $cm^3 \times mol^{-1}$	δ1/2	HLB_R	t_K °$C_{(x)}$	$\eta \times 10^{-4}$ P	α_{Vo} 10^{-5} K^{-1}	α_{Vo} 10^{-5} K^{-1}
1. Cholic acid · n_{TE} = 10	78481.8	690.58	10.6604	23.7493	37.50	33.9404	7.6641	2.5547
2. Cholic acid · n_{TE} = 20	120478.2	1169.02	10.1518	21.1290	33.00	30.6801	7.7770	2.5923
3. Cholic acid · n_{TE} = 30	139785.8	1388.98	10.0319	20.5443	36.35	29.9328	7.6928	2.5642
4. Cholic acid · n_{TE} = 40	180170.6	1849.06	9.8711	19.7795	43.15	28.7983	7.5275	2.5091
5. Cholic acid · n_{TE} = 50	203712.6	2117.26	9.8089	19.4895	50.35	28.4544	7.3599	2.5091
6. Cholic acid · n_{TE} = 60	226369.8	2375.38	9.7621	19.2731	47.35	27.7697	7.4288	2.4762
7. Cholic acid · n_{TE} = 70	277846.2	2961.82	9.6855	18.9233	53.15	26.9851	7.2968	2.4322

Table 5. Determined by means ^{1}H NMR, content of oxyethylated segments and the level of hydrophilic-lipophilic balance (HLB), along with selected physicochemical characteristics of the products of cholic acid oxyethylation calculated based on the ^{1}H NMR parameters.

Cholic acid oxyethylation product	Content of n_{TE}	M_w	E (w%)	Griffin method	HLB Davies method	^{1}H NMR	H/L
1. Cholic acid · n_{TE} = 10	10.48	870.25	53.04	10.61	14.45	12.25	1.0861
2. Cholic acid · n_{TE} = 20	23.77	1455.72	71.93	14.38	18.83	12.85	2.6921
3. Cholic acid · n_{TE} = 30	29.88	1724.88	76.31	15.26	20.85	13.14	2.9220
4. Cholic acid · n_{TE} = 40	42.66	2287.88	82.14	16.43	25.06	15.01	2.3687
5. Cholic acid · n_{TE} = 50	50.11	2616.07	84.38	16.87	27.52	16.59	3.6921
6. Cholic acid · n_{TE} = 60	57.28	2931.93	86.06	17.21	29.89	17.79	6.0848
7. Cholic acid · n_{TE} = 70	73.57	3649.55	88.80	17.76	35.26	19.22	7.0881

Table 6. Basic viscosity and hydrodynamic parameters of the methanol-soluble products of cholic acid oxyethylation.

Cholic acid oxyethylation product	c_{exp} g × 100 cm^{-3}	GLL; [η]	M_η	$R_o \times 10^{-7}$cm	$R_{obs.} \times 10^{-8}$ cm	$\Omega \times 10^{-20}$ cm^3	$n_{/s/}CH_3OH$*
1. Cholic acid · n_{TE} = 10	2.0360	0.079757	2559.93	4.02708	3.2880	1.4890	52.73
2. Cholic acid · n_{TE} = 20	2.0870	0.072562	2020.76	3.7389	3.0527	1.1917	17.63
3. Cholic acid · n_{TE} = 30	2.1512	0.068951	1778.44	3.2932	2.6889	0.8143	1.64
4. Cholic acid · n_{TE}= 40	2.2534	0.0795719	2545.09	3.8927	3.1783	1.3449	8.02
5. Cholic acid · n_{TE} = 50	2.6224	0.0921391	3673.12	4.6195	3.7717	2.2476	32.99
6. Cholic acid · n_{TE}= 60	3.3418	0.0942007	3882.19	4.7403	3.8704	2.4287	29.65
7. Cholic acid · n_{TE} = 70	2.5296	0.1076327	5418.90	5.5384	4.5220	3.8734	55.22

Table 7. Basic viscosity and hydrodynamic parameters of the water-soluble products of cholic acid oxyethylation.

Cholic acid oxyethylation product	c_{exp} g × 100 cm^{-3}	GLL; [η]	M_η	$R_o \times 10^{-7}$cm	$R_{obs.} \times 10^{-8}$ cm	$\Omega \times 10^{-20}$ cm^3	$n_{/s/}CH_3OH$*
1. Cholic acid · n_{TE} = 20	1.1278	0.088188	1981.69	3.7061	3.0259	1.1606	29.15
2. Cholic acid · n_{TE} = 30	1.2001	0.085357	1877.50	3.6006	2.9398	1.0643	8.47
3. Cholic acid · n_{TE} = 40	1.2049	0.091555	2108.52	3.8311	3.1280	1.2820	-
4. Cholic acid · n_{TE}.= 50	1.2679	0.107031	2730.71	4.3991	3.5917	1.9410	6.36
5. Cholic acid · n_{TE} = 60	1.1640	0.105401	2662.15	4.3397	3.5432	1.8634	-
6. Cholic acid · n_{TE}= 70	1.4387	0.113540	3011.11	4.6351	3.7844	2.2705	-

Table 7. Basic viscosity and hydrodynamic parameters of the water-soluble products of cholic acid oxyethylation.

Cholic acid oxyethylation product	c_{exp} g × 100 cm^{-3}	GLL; [η]	M_η	$R_o \times 10^{-7}$cm	$R_{obs.} \times 10^{-8}$ cm	$\Omega \times 10^{-20}$ cm^3	$n_{/s/}H_2O$
1. Cholic acid · n_{TE} = 20	1.1278	0.088188	1981.69	3.7061	3.0259	1.1606	29.15
2. Cholic acid · n_{TE} = 30	1.2001	0.085357	1877.50	3.6006	2.9398	1.0643	8.47
3. Cholic acid · n_{TE} = 40	1.2049	0.091555	2108.52	3.8311	3.1280	1.2820	-
4. Cholic acid · n_{TE}.= 50	1.2679	0.107031	2730.71	4.3991	3.5917	1.9410	6.36
5. Cholic acid · n_{TE} = 60	1.1640	0.105401	2662.15	4.3397	3.5432	1.8634	-
6. Cholic acid · n_{TE}= 70	1.4387	0.113540	3011.11	4.6351	3.7844	2.2705	-

RESULTS AND DISCUSSION

In order to assess the quality of products of the catalytic oxyethylation of cholic acid, their chromatographic parameters (determined by HPLC and GPC) were compared to the reference Tween type polysorbates (Tween-40, -60, -61, -80, -85) provided by world-leading manufacturers of this class of non-ionic surfactants (22-24).

Surprisingly, the Tween type polysorbates (Table 3) were characterized by a marked dispersion of molecular masses (M_h i M_w) and high contents of polyethylene glycols (PEG) ranging from 22.33 to 44.22% by a declared number of oxyethylated segments (n_{TE} = 20); the only exception pertained to the Tween-61 type polysorbate whose $n_{TE} \cong 4$.

However, the polyethylene glycol content was variable in the case of the homologous cholic acid oxyethylation products with n_{TE} ranging from 10 to 70. Determined content of PGE ranged from 10.62 to 42.52% for the product of cholic acid with n_{TE} = 50 and the product with n_{TE} = 70, respectively.

Physicochemical values characterizing the structure of the products of oxyethylation of cholic acid with the progressive content of ethylene oxide (n_{TE}) presented in Table 4 suggest that the process of liquefaction takes place within a relatively narrow range of drop-point temperatures (t_K, ^oC) from 37.5 to 53.5^oC. This finding actually determines the direction of their application in drug form technology. Interestingly, an increase in the content of hydrophilic oxyethylated segments (n_{TE}) in the product structure was associated with the systematic decrease in structural viscosity in the state of liquefaction ($\eta \times 10^{-4}$ P) and the lower calculated values of the volume expansion coefficient (α_{vo}) as well as the linear expansion coefficient (α_{lo}). This suggests the anticipated increase in the water solubility of the product and substantiates its application as a solubilizer for lipophilic BCS Class II and III therapeutic agents (5-8, 10).

The use of the selective catalyst (NaOH) in the process of oxyethylation does not necessarily correspond to symmetric addition of ethylene oxide to hydroxyl groups remaining in the structure of cholic acid molecule.

Consequently, the content of n_{TE} determined by means of ^{1}H NMR corresponds to the total participation of hydrophilizing factor in analyzed product. Therefore, this content in Table 5 constituted the basis for estimating the weight-average molecular mass (M_w) and particularly for determining the level of hydrophilic-lipophilic balance (HLB) in independent notations (Griffin, Davies, ^{1}H NMR i HLB_R methods) and calculating the solubility parameter – $\delta^{1/2}$ in Tables 4 and 5.

The oxyethylation products with HLB ≥ 14 (as determined by the Griffin method) were characterized by satisfactory solubility in Table 2. Consequently, such products may be used during preformulation research as micellar solubilizers for lipophilic therapeutic agents (BCS Class II and III) and cholesterol (HLB = 1.0).

Hydrodynamic parameters of the micelle solubilizing lipophilic therapeutic agents in equilibrium conditions

Determined by means of the Ubbelholde method, the viscosity of aqueous solutions of the cholic acid oxyethylation products with n_{TE} = 20 enabled calculation of the limiting viscosity number (LVN [η]), the content-average molecular mass (M_η) and the hydrodynamic values of the micelle, i.e., R_o, R_{obs}, and Ω in Table 6.

Moreover, the number of the water molecules bound in the pallisadic layer of the micelle by hydrophilic segments of solubilizers in Table 7 was determined from the equation:

$$n_{H_2O} = \frac{M_\eta - M_w}{18.015}$$

Despite its interpretational complexity, the value of n_{H_2O} describes the depth of the hydration layer. In turn, the thermodynamic stability of the latter determines the mechanism and solubilization site of the lipophilic therapeutic agent.

Furthermore, the number of methanol molecules solvated by the lipophilic "core" of the oxyethylation product in Table 6 was estimated based on the following formula:

$$n_{/s/}CH_3OH = \frac{M_\eta - M_w}{M_{cz}CH_3OH}$$

The basic viscosity (hydrodynamic) parameters characterizing the efficiency and the site of solubilization of selected lipophilic therapeutic agents are summarized in Tables 8-10. These data demonstrate that from a thermodynamic viewpoint the process of micellar adduct formation is not associated with the loss of water molecules from the pallisadic layer of a micelle. This finding corresponds to an increase in hydrodynamic parameters of the micelle of the adduct (R_o, R_{obs}, Ω), specific for the analyzed therapeutic agent, and is further confirmed by the solubilization index value which is calculated from the following formula:

$$n_{/s/} = \frac{M_{\eta_{adduct}} - M_{w_{solubilizer}}}{M_{w_{ther.\ agent}}}$$

Table 8. Basic viscosity and hydrodynamic parameters of the aqueous solutions of the cholic acid oxyethylation products solubilizing diclofenac micellarly and quantitatively.

Cholic acid oxyethylation product	c_{exp} g × 100 cm^{-3}	GLL; [η]	M_η	$R_o \times 10^{-7}$cm	$R_{obs.} \times 10^{-8}$cm	$\Omega \times 10^{-20}$ cm^3	C_D solubilized diclofenac*
1. Cholic acid · n_{TE} = 20	1.1278	0.0840446	1829.92	3.5515	2.8999	1.0213	15.5259 mg/100 cm^3
2. Cholic acid · n_{TE}.= 30	1.2001	0.0864516	1917.51	3.6414	2.9731	1.1009	18.7353 mg/100 cm^3
3. Cholic acid · n_{TE} = 40	1.2049	0.1058291	2680.12	4.3553	3.5560	1.8836	12.5389 mg/100 cm^3
4. Cholic acid · n_{TE}.= 50	1.2679	0.117749	3198.14	4.7869	3.9084	2.5009	14.0864 mg/100 cm^3
5. Cholic acid · n_{TE} = 60	1.1640	0.1301482	3774.71	5.2305	4.2706	3.2626	5.7387mg/100 cm^3
6. Cholic acid · n_{TE}.= 70	1.4387	0.1456743	4549.03	5.7792	4.7186	4.4009	5.1668 mg/100 cm^3

Table 9. Basic viscosity and hydrodynamic parameters of the aqueous solutions of the cholic acid oxyethylation products solubilizing loratadine micellarly and quantitatively.

Cholic acid oxyethylation product	c_{exp} g × 100 cm^{-3}	GLL; [η]	M_η	$R_o \times 10^{-7}$cm	$R_{obs.} \times 10^{-8}$cm	$\Omega \times 10^{-20}$ cm^3	$n_{f/}$ loratadine*
1. Cholic acid · n_{TE} = 20	1.1278	0.0865422	1920.83	3.6448	29759	1.1039	1.2147
2. Cholic acid · n_{TE} = 30	1.2001	0.0778498	1612.05	3.3188	2.7097	0.8334	-
3. Cholic acid · n_{TE} = 40	1.2049	0.0856568	1888.40	3.6117	2.9489	1.0722	-
4. Cholic acid · n_{TE} = 50	1.2679	0.1015223	2507.96	4.2014	3.4303	1.6909	-
5. Cholic acid · n_{TE} = 60	1.1640	0.1275324	3649.94	5.1373	4.1945	3.0914	1.8752
6. Cholic acid · n_{TE} = 70	1.4387	0.1404246	4280.83	5.5945	4.5677	3.9922	1.6487

Table 10. Basic viscosity and hydrodynamic parameters of the aqueous solutions of the cholic acid oxyethylation products solubilizing rutin (rutoside) micellarly and quantitatively.

Cholic acid oxyethylation product	c_{exp} g × 100 cm^{-3}	GLL; [η]	M_η	$R_o \times 10^{-7}$cm	R_{obs}, × 10^{-8} cm	Ω × 10^{-20} cm^3	C_R^*, amount of solubilized rutin (mg × 100 cm^{-3})	
							C_1	C_2
1. Cholic acid · n_{TE} = 20	1.1278	0.1341835	3970.45	5.3738	4.3876	3.5382	86.7591	86.2595
2. Cholic acid · n_{TE} = 30	1.2001	0.1162842	3132.53	4.7341	3.8653	2.4191	54.5879	53.9758
3. Cholic acid · n_{TE} = 40	1.2049	0.1338715	3955.17	5.3627	4.3785	3.5164	61.4025	60.8142
4. Cholic acid · n_{TE} = 50	1.2679	0.1324055	3883.72	5.3107	4.3361	3.4151	49.1204	48.4891
5. Cholic acid · n_{TE} = 60	1.1640	0.1567291	5134.66	6.1658	5.0342	5.3445	48.8035	48.1711
6. Cholic acid · n_{TE} = 70	1.4387	0.1513164	4844.42	5.9795	4.8822	4.8747	64.8891	64.3129
7. Water (H_2O)							25.8779	25.8079

Moreover, this aforementioned observation was confirmed by the amounts of solubilized therapeutic agents: diclofenac, loratadine and rutin in Tables 8-10.

Basic calculated viscosity and hydrodynamic parameters along with spectroscopically determined amount of solubilized diclofenac, loratadine and rutin suggest that the thermodynamically stable adduct is synthesized as a result of the equilibrium process of solubilization. The effective volume (Ω) of this adduct is the function of the amount of therapeutic agent adsorbed in the pallisadic layer of the micelle structure.

These aforementioned parameters are important for potential, effective *in vivo* transport of therapeutic agent (analogically to the processes observed in human bile A).

CONCLUSIONS

Measuring the drop-point temperature of the products of oxyethylation in the presence of the selective catalyst (NaOH) of cholic acid suggested a further direction of research concerned with their application in drug form technology. This was possible due to the estimation of the structural viscosity in the state of equilibrium liquefaction (η) and the volume and linear expansion coefficients (α_{Vo} and α_{lo}). An increase in the hydrophilic properties of the product structure was revealed to be reflected by a systematic decrease in structural viscosity (η) and expansion coefficients (α_{Vo} i α_{lo}). Interestingly, the estimated drop-point temperature for the homologous series of products ranged from 37.5 to 53.5^{o}C. These aforementioned properties enable the application of the selected derivative as a solubilizer compatible with the composition of bile A. Such a solubilizer can be used in the solid oral dosage form with a lipophilic therapeutic agent.

As determined by means of ^{1}H NMR, the content of oxyethylated segments in the cholic acid n_{TE} = 10 - 70 type product enabled the verification of the weight-average molecular mass (M_w) and particularly the estimation of the analytic level of hydrophilic-lipophilic balance (HLB) in independent notations. The values of HLB suggest that the application of catalyst (NaOH) is reflected by satisfactory water solubility and expected solubilizing properties of the cholic acid n_{TE} = 20 type products of oxyethylation. Viscosity measurements enabled the calculation of selected hydrodynamic parameters of the micelle (R_o, R_{obs}, Ω, and $n_{/s/}$) and the adduct following the process of equilibrium solubilization. The values summarized in Tables 8-10 demonstrate that the

process of the lipophilic therapeutic agent solubilization does not disturb the hydration layer of the micelle. This was reflected by the individual increase in the hydrodynamic radius (R_{obs}) and the effective volume (Ω) of the adduct's micelle. Satisfactory solubilizing properties of selected products of the cholic acid oxyethylation in Tables 8-10 were also confirmed by the amounts of solubilized in equilibrium lipophilic therapeutic agents (BCS Class II and III) that were determined spectrometrically.

The results of this preformulation research suggest that the products of cholic acid oxyethylation with symmetric addition of ethylene oxide to hydroxyl groups are characterized by significant quantitative solubilizing properties with respect to selected lipophilic therapeutic agents.

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