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In vitro Release Characteristics of Nitroglycerin from Microemulsion-Based Hydrogel System for Anal Fissure Treatment

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ABSTRACT – To develop topical nitroglycerin (NTG) preparation for chronic anal fissure treatment, the release rate of NTG should be controlled carefully. For this, microemulsion was prepared from the phase diagram construction with Cremophor ELP®, ethanol and Labrafil M1944CS® and the topical gel was prepared by dispersing NTG containing microemulsion into hydrophilic polymers. *In vitro* release characteristics were evaluated with Franz diffusion cell using cellulose membrane and compared with control hydrogels. The release rate of NTG was followed 1st order kinetics and ,when comparing the NTG release from control hydrogel with that from the microemulsion-based hydrogel, the NTG release rate was controlled by the content of polymers within continuous phase and the concentration of dispersed phase.

Key words – Nitroglycerin, Mictroemulsion, Hydrogel, Controlled release, Anal fissure

Nitroglycerin (Propane-1, 2, 3-triol trinitrate; NTG) (Figure 1) has been used for the treatment of angina pectoris,[1] heart failure[2] and myocardial infarction.[3] NTG is also known as glyceryl trinitrate or trinitrin,[4,5] and the organic nitrates generate nitric oxide (NO) known as a membrane permeable, lipophilic gas that is found in endothelial cells, platelets, neutrophils, and the brain.[6] The NO increases the blood cyclic guanosine monophosphate (cGMP) production and leads to decrease in blood pressure.[7]

Recently, NO donor sources such as NTG have been extensively studied also in the chronic anal fissure treatment.[8] The general pathogenesis of anal fissure is suggested as an ischemic ulcer caused by the combination of spasm in the internal anal sphincter and relative ischemia in the posterior midline of the anal canal.[9] From Utzig[10] and Lindsey,[11] it was revealed that NTG could decrease anal tone about 25%-30%, and has been shown to increase anodermal blood flow without permanent disruption of the normal sphincter function. Topical NTG may diffuse across the cutaneous barrier, causes a reduction of internal anal sphincter pressure, and improves anodermal blood flow.

To develop topical NTG formulation, NTG solubilizing component should be considered because NTG is slightly soluble in the water media.[12] Moreover, it is a very crucial point to prevent side effects, which were related with the amount of administered NTG dose,[13] such as headache, hypotention, marked slowing of the heart rate and syncope by controlling the absorption rate of NTG. In these background, NTG sustained releasing formulation is required for developing safety guaranteed topical NTG delivery system.

Generally, the microemulsion system has been known to increase the skin penetration of incorporated drugs.[14,15] However, by Špiclin, ascorbyl palmitate was solubilized and the release rate was controlled by the viscosized microemulsion.[16] Moreover, the release rate of indomethacin from lecithin-based microemulsions could be controlled depends on the colloidal microstructure of microemulsion.[17] In other words, the drug release characteristics control is possible by regulating the composition of continuous phase or internal structure of microemulsion.

In this research, we formulated NTG into microemulsion system. NTG was solubilized within the internal oil phase and dispersed into hydrogel base and we observed the release characteristics of NTG from microemulsion-based hydrogel system by comparing with control hydrogels.

Experimental

Materials

Nitroglycerin 1% solution was purchased from Merck Co. (Germany). Sodium alginate and sodium CMC were obtained

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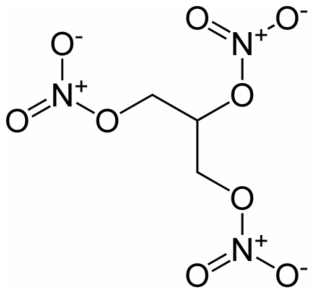

Figure 1–Chemical structure of nitroglycerin.

from Yakuri pure chemical Co. (Japan), Labrafil M1944CS® and Cremophor ELP® was obtained from Gatteffossè (France) and BASF (Germany), respectively. The cellulose membrane was purchased from Spectrum laboratories (Spectra/Por®, U.S.A. and all other solvents reagents were of reagent grade and used as received.

Preparation of phase diagram

To make NTG containing microemulsion, phase diagram was constructed first. Cremophor ELP®, ethyl alcohol and Labrafil M1944CS® was selected as surfactant, co-surfactant and oil, respectively. The ratio of Cremophor ELP® to ethyl alcohol was fixed as 2 : 1 and Labrafil M1944CS® was added to the mixture of Cremophor ELP® and ethyl alcohol with a various ratio. Finally, distilled water was added to the microemulsion pre-concentrate and the area was defined as follows; M : microemulsion, E : emulsion, Tp : separated two phase, Tu : turbid gray solution and Tr : transparent monophase.

Preparation of NTG control hydrogel

NTG was solubilized and diluted with ethanol carefully in hood to make provision for the expolsion. Water was added to the NTG ethanol solution and hydrophilic polymers were added to the NTG solution and mixed slowly for 6 h. The final NTG content was 0.05% (Table I).

Preparation of NTG microemulsion-based hydrogel

From the phase diagram, the ratio of Labrafil M1944CS® was fixed as 25% which passed the centre of microemulsion area. Cremophor ELP®, ethyl alcohol were mixed and NTG was added to the mixture of Cremophor ELP® and ethyl alcohol. To this, Labrafil M1944CS® was added and the final NTG microemulsion pre-concentrate was mixed with water media. Finally, microemulsion-based hydrogel was prepared by adding hydrophilic polymer (Table II, III).

Table I–*The Composition of Nitroglycerin Control Hydrogel*

Ingredient	Cont-SA gel	Cont-SC gel
Ethanol	19.95	19.95
Nitroglycerin	0.05	0.05
Sodium alginate	2.50	-
Sodium CMC	-	3.50
Water	77.50	76.00
Total	100.00	100.00

Table II–*The Composition of Nitroglycerin Microemulsion-based Hydrogel with Different Polymer Content*

Ingredient	M-SA2	M-SA3	M-SA4	M-SC2.5	M-SC3.5	M-SC4.5
Ethanol	4.95	4.95	4.95	4.95	4.95	4.95
Cremophor ELP	10.00	10.00	10.00	10.00	10.00	10.00
Labrafil M1944CS	5.00	5.00	5.00	5.00	5.00	5.00
Nitroglycerin	0.05	0.05	0.05	0.05	0.05	0.05
Sodium alginate	2.00	3.00	4.00	-	-	-
Sodium CMC	-	-	-	2.50	3.50	4.50
Water	78.00	77.00	76.00	77.50	76.50	75.50
Total	100.00	100.00	100.00	100.00	100.00	100.00

***In vitro* NTG release study**

The NTG release characteristic was studied with Franz-diffusion cell system. The effective diffusion area was 1.76 cm^2 and 0.8 gram of hydrogel samples were loaded onto donor compartment of Franz diffusion cells. pH 7.4 PBS containing 30% ethanol was used as a receptor solution (11 mL) and maintained at 37 ± 0.5.

Considering that the NTG formulation will be applied to the partially damaged or open wound skin of anal fissure in clinical field, the release characteristic is more important evaluation point than skin penetration. In this reason, we used cellulose membrane having 12,000 daltons of molecular cut off enough for the free diffusion of NTG to evaluate and compare the release characteristics of NTG from microemulsion-based hydrogel system.

Measurement of viscosity of gel

The viscosity was measured with a viscometer (Haake RV100, Germany) and the measurements were made at room temperature.

Measurement of particle size

The diameter of microemulsion was determined using dynamic light scattering system (LPA PARIII, Ostuka Electronics, Japan) at 25°C, scattering angle of 90° and 12,000 count per sec.

Table III–*The Composition of Nitroglycerin Microemulsion-based Hydrogel with Different Amount of Microemulsion Preconcentrate*

Ingredient	MPC20	MPC25	MPC30	MP35C	MPC40	MPC20	MPA25	MPA30	MPA35	MPA40
Ethanol	5.00	6.20	7.50	8.70	10.00	5.00	6.20	7.50	8.70	10.00
Cremophor ELP	10.00	12.50	15.00	17.50	20.00	10.00	12.50	15.00	17.50	20.00
Labrafil M1944CS	5.00	6.30	7.50	8.80	10.00	5.00	6.30	7.50	8.80	10.00
Nitroglycerin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium alginate	2.00	2.00	2.00	2.00	2.00	-	-	-	-	-
Sodium CMC	-	-	-	-	-	2.50	2.50	2.50	2.50	2.50
Water	77.95	72.5	67.95	62.95	57.95	78.00	73.00	68.00	63.00	58.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

HPLC analysis condition

Reverse phase m-Bondapak (C_{18}, 4.6×150 mm, 5 μm, Waters, USA.) column was used for the analysis of NTG. The composition of mobile phase was methanol : water=56 : 44 (v/v) and the flow rate was 1.0 mL/min. Detection wavelength and injection volume was 204 nm and 20 μL, respectively.

Results and Discussion

In phase diagram construction study, the microemulsion area was found when the portion of oil occupies 20%~70% of microemulsion pre-concentrate and the amount of added water was over 50% of total mixture (Figure 2). The particle size of microemulsion was decreased when the water content was increased and the particle size was under 100 nm when the water content was over 70% (Figure 3). From these results, it was possible to prepare stable, fine microemulsion below 100 nm.

In the *in vitro* release test with control hydrogels, the NTG released apparently 1st order pattern until 12 h, but no statistical significant difference in the NTG release pattern was observed by the kind of polymers (Table I, Figure 4).

In the viscosity measurement test, the viscosity of sodium alginate increased from 4,000 cps to 44,000 cps when the content of

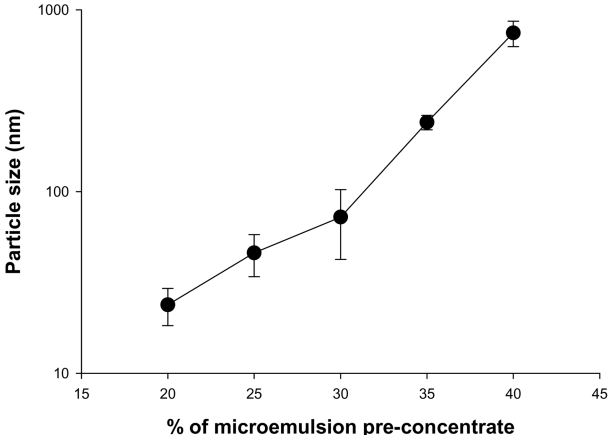

Figure 3–The particle size of microemulsion by the change of concentration of microemulsion pre-concentration.

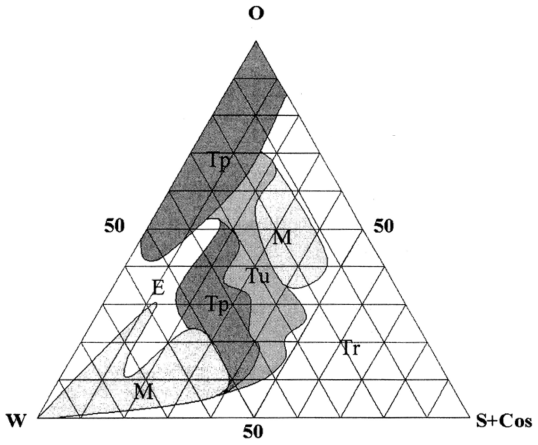

Figure 2–Phase diagram of microemulsion system: M : microemulsion, E : emulsion, Tp : separated two phase, Tu : turbid gray solution and Tr : transparent monophase.

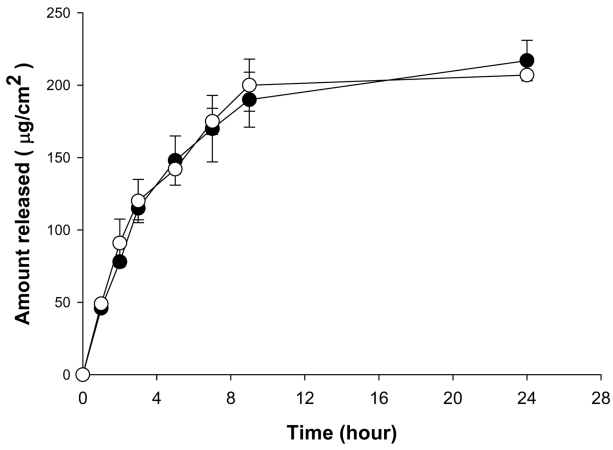

Figure 4–Nitroglycerin release profile from control hydrogels. ●; sodium alginate, ○; sodium CMC. Data are expressed as mean S.E. ± (n=3).

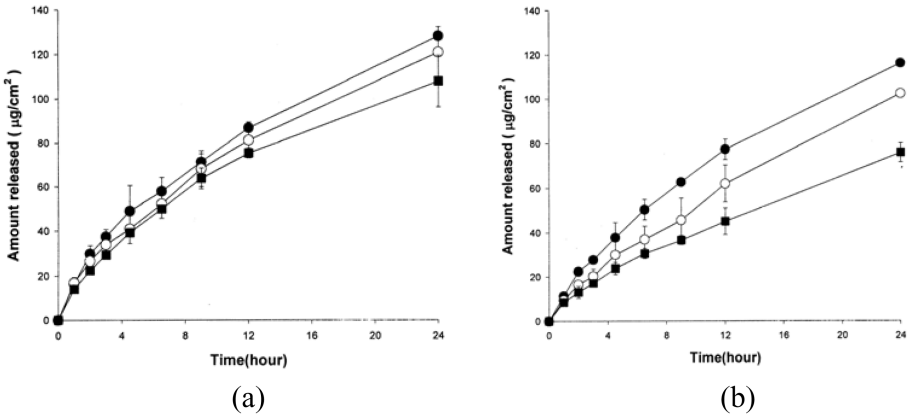

Figure 5—Nitroglycerin release profile from microemulsion-based hydrogel. (a) The in vitro release profile of nitroglycerin was compared with the change of sodium alginate content. ●; 2%, ○; 3%, ■; 4%. (b) The in vitro release profile of nitroglycerin was compared with the change of the sodium CMC content. ●; 2.5%, ○; 3.5%, ■; 4.5%. Data are expressed as mean ± S.E. (n=3).

sodium alginate was increased from 2% to 4%. In case of sodium CMC, the viscosity was changed from 5,500 cps to 60,200 cps when the content of sodium CMC was increased 2.5% to 4.5% (Table IV). In the in vitro release study with microemulsion-based hydrogel after fixing the components of microemulsion, NTG released as 1st order fashion also and the release of NTG was controlled by the content of polymer within continuous phase and the release rate was reciprocally proportion to the content of hydrophilic polymer (Table II, Figure 5).

Table IV—*The Viscosity Change of Nitroglycerin Hydrogel*

	Sodim alginate (%)			Sodium CMC (%)		
	2	3	4	2.5	3.5	4.5
Viscosity (cps)	4,000	16,000	44,000	5,500	24,500	60,200

In vitro release of NTG was compared by changing the concentration of the dispersed phase (Table III, Figure 6). The release rate was decreased when the amount of microemulsion pre-concentrate was increased. And no significant differences were found between among different formulations of sodium alginate and sodium CMC hydrogel. Gasco et al has reported that the microemulsion could be a drug reservoir and the reservoir effect will be more increased when the incorporated drug has oil soluble characteristics.[18] Trotta et al also reported that the drug solubilized within the internal oil phase of microemulsion will be released first into the continuous phase and the drug release will be controlled again by the characteristics of continuous phase.[19] Comparing the control hydrogel with microemulsion-based hydrogel, it was obvious that the NTG release was controlled by the microemulsion system.

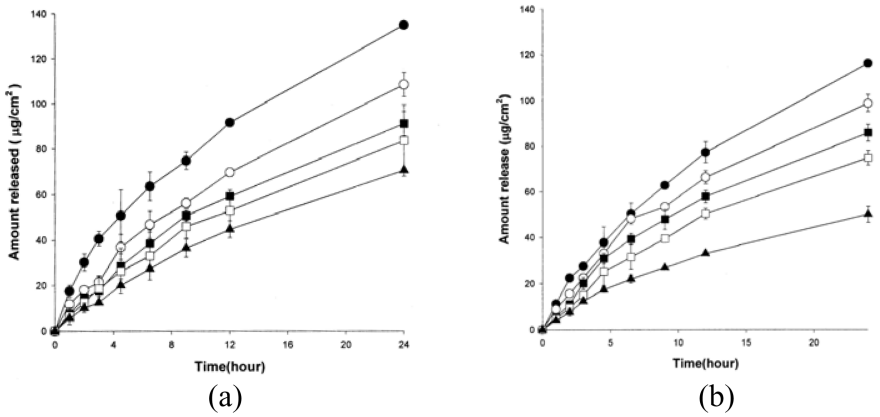

Figure 6—(a) Nitroglycerin release profile from microemulsion-based sodium alginate hydrogel. The content of sodium alginate was fixed at 2% and the in vitro release profile of nitroglycerin was compared with the change of microemulsion pre-concentration content. ●; 20%, ○; 25%, ■; 30%, □; 35%, ▲; 40%. (b) Nitroglycerin release profile from microemulsion-based sodium CMC hydrogel. The content of sodium CMC was fixed at 2.5% and the in vitro release profile of nitroglycerin was compared with the change of microemulsion pre-concentration content. ●; 20%, ○; 25%, ■; 30%, □; 35%, ▲; 40%. Data are expressed as mean ± S.E. (n=3).

From this, it was considered that the main NTG release controlling factor is the diffusion rate of NTG from microemulsion, because control hydrogel didn't show the controlling effect of NTG release (Figure 4).

Conclusion

Microemulsion system is known as a very fine, thermodynamically stable drug delivery system. This system is also attractive because of its solubilizing ability of water insoluble drug and enhancing the skin permeation of drugs.[20,21] However, as we can see in this study, the release characteristics of NTG could be controlled by the content of polymer within continuous phase and concentration of the dispersed phase. To sum up, the main mechanism of NTG release control is owing to the release rate controlling effect of oil phase of microemulsion because the more the amount of oil of microemulsion increases, the thicker the radius of oil core and the lower release rate from the oil phase to gel matrix. From this, it was expected that the present NTG microemulsion-based hydrogel system will be a good candidate for the treatment of anal fissure by lowering the occurrence of side effects induced by fast absorption of NTG.

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