Vitamin K_1 Purification Process in a Molecular Evaporator

[a]J. CVENGROŠ*, [b]M. MRÁZEK, and [b]G. KMETTY

[a]*Department of Physical Chemistry, Faculty of Chemical Technology, Slovak University of Technology, SK-812 37 Bratislava*
e-mail: cvengros@chtf.stuba.sk

[b]*Slovakofarma, AG, SK-920 27 Hlohovec*

Received 23 February 1998

A purification process of crude phytomenadione (vitamin K_1) by molecular distillation using a short-path wiped-film evaporator and by adsorption is studied. The purification procedure is documented by evaluation of samples from the individual stages of the process by means of UV and visible spectroscopies, TLC, refraction index measurements, and further methods. Satisfactory parameters were found in a material obtained as the distillate from the molecular evaporator, particularly after its adsorption on a phenolformaldehyde-resin-based ion exchanger.

Phytomenadione (vitamin K_1) is 2-methyl-3-phytyl-1,4-naphthoquinone, $M = 450.7$ g mol^{-1}. It belongs to a group of methylnaphthoquinone derivatives exhibiting antihaemorrhagic effects. Their side chains consist of several isoprene units. Vitamin K_1 is a yellow to red-yellow oily liquid easily soluble in common organic solvents, less soluble in ethanol and methanol, and insoluble in water. The compound is stable in slightly acidic media, under oxidizing conditions, in moisture and heat, but is very sensitive to UV radiation and alkaline medium. It can be synthesized by condensation of 2-methyl-1,4-naphthoquinone with phytol to form 2-methyl-3-phytyl-1,4-naphthoquinone as an intermediate which is then oxidized to phytomenadione in the presence of silver oxide.

For quantitative determination, UV spectra are taken. The values of light absorption, or their ratios in the λ-range 220 to 350 nm serve as a purity criterion. According to *British Pharmacopoeia* [1], the UV spectrum of phytomenadione in 0.002 % (w/v) solution in 2,2,4-trimethylpentane shows four maxima at 243 nm, 249 nm, 261 nm, and 270 nm. Their respective absorbance values are about 0.80, 0.84, 0.77, and 0.78. The spectrum also exhibits minima at 228 nm, 246 nm, 254 nm, and 266 nm. The ratio of the absorbance at the minimum at 254 nm to that at the maximum of 249 nm is ranging from 0.70 to 0.75. Moreover, the 0.02 % (w/v) phytomenadione solution in 2,2,4-trimethylpentane exhibits a maximum at 327 nm and a minimum at 285 nm within the interval of 230 nm to 350 nm. The absorbances at 327 nm and 285 nm are about 1.4 and 0.44, respectively.

To purify phytomenadione, especially chromatographic methods are applied [2]. Molecular distillation as a method for final treatment of raw K_1 is also mentioned [3, 4], giving no further details. The absence of the study on possibilities and results of this effective method in relation to the vitamin K_1 led us to working out the submitted paper.

Molecular distillation is generally accepted as the most preserving distillation method to separate and purify both thermolabile higher-molecular-mass compounds and low-vapour-pressure liquid mixtures without decomposing them thermally. It has the following fundamental features:

– a short residence time of the distilled liquid on the heated surfaces;

– a decreased working temperature as a result of high vacuum inside the distillation apparatus;

– a characteristic mass transfer mechanism in the gap between the evaporator and condenser.

The short residence time of the liquid from several to tens of seconds can be reached by spreading the liquid into a thin wiped film of a thickness from 0.05 to 0.5 mm depending on its viscosity and the feed. Compared to the normal pressure process, removing the gas barrier above the evaporation surface at a low pressure from 10^{-1} to 10^{1} Pa will decrease the operational temperature by up to 250 ℃. If the exposition time is short and temperature substantially decreased, the yield of decomposition reactions is reduced to a negligible value and, therefore, the process is going virtually without any thermal decomposition. The short distance between the evaporation and con-

*The author to whom the correspondence should be addressed.

densation surfaces from 10 to 50 mm in combination with a low pressure allows a high fraction of the evaporated molecules to reach the cooled surface and to condense on it without being hindered. This will ensure a high distillation rate up to 40 g m^{-2} s^{-1} which might be interesting even for industrial applications.

Owing to its properties, the crude vitamin K_1 is suitable for applying this separation method in order to be finally purified.

The aim of this study is to demonstrate the phytomenadione purification process in molecular evaporator in its individual steps using particularly UV spectroscopy as the detection method of purification efficiency. Other methods were also used for this purpose, *e.g.* VIS spectroscopy, TLC, and refractometry.

EXPERIMENTAL

Molecular Evaporator

The distillation of crude phytomenadione (Ph) was performed using two small wiped-film MO30 molecular evaporators (Faculty of Chemical Technology, Slovak University of Technology, Bratislava). The MO30 evaporator works continuously, has a convex evaporation surface located on the outer surface of the evaporation cylinder of a 30 mm diameter coaxially surrounded with the condenser. The distance between evaporation and condensation surfaces is 25 mm. The evaporation surface is 280 cm^2, the optimum feed rate is about 1 dm^3 h^{-1}, the operational temperature up to 300 ℃, and the pressure inside the evaporator is ranging from 10^{-1} to 10^1 Pa. The evaporator is made of glass. A detailed description of the MO30 evaporator is given in [5].

Spectral Measurements

UV and VIS spectra were measured with a Philips 873 spectrometer (Philips, Cambridge, England). Its spectral resolution was 2 nm and optical sensitivity ± 0.1 nm.

Individual samples were dissolved in 2,2,4-trimethylpentane to form both 0.002 % (w/v) and 0.02 % (w/v) solutions and their spectra in the λ-range 220 to 350 nm were measured. For measuring absorbance in the visible region at 550 nm, solventless samples against empty cells were used. Cell thickness was 1 cm.

Ph content in the samples was determined from the absorbance of the solution prepared by dissolution of 0.1 g sample in 2,2,4-trimethylpentane to obtain 100 cm^3 of the solution. 10 cm^3 of this solution were then diluted with 2,2,4-trimethylpentane to get the total volume of 100 cm^3 and the same solvent was added to 10 cm^3 of the latter solution to obtain the total volume of 100 cm^3. The absorbance of the final solution was measured against the solvent at the maximum at 249 nm. The Ph content was calculated from the relation $A_{\mathrm{sample}} \times 1000/A(1\ \%, 1\ \mathrm{cm})$ where $A(1\ \%, 1\ \mathrm{cm}) = 410$ at the maximum at 249 nm.

Crude Phytomenadione Purification

The crude phytomenadione was degassed using the first molecular evaporator at the heating medium temperature of 130 ℃ and at a pressure ranging from 1 to 3 Pa. The distillation residue during the second passage through the first evaporator was processed at the heating oil temperature of 180 ℃ and at a pressure ranging from 0.8 to 2 Pa. The feed rate was 0.7 dm^3 h^{-1}. Under these conditions, the first fraction containing about 6 % of the feed and a distillation residue were obtained. The distillation residue was then distilled using the second molecular evaporator at the heating oil temperature of 198 ℃ and at a pressure ranging from 0.3 to 0.6 Pa at a feed rate 0.7 dm^3 h^{-1}. The distillate containing 82 to 85 % of the total processed quantity was the distilled Ph which was finally purified by adsorption on weak basic anion-exchange resin DUOLITE® A561 (Rohm and Haas, Paris, France). The distilled Ph was diluted with heptane at the volume ratio $\varphi_r = 20 : 3$ and then fed into the adsorption column at a rate of 4 dm^3 h^{-1}. Elution with heptane at a rate of 20 dm^3 h^{-1} took about 5 h. The solvent was removed from the eluate using a vacuum rotary evaporator. All these operations and tests were performed in a dark room in subdued light.

Thin-Layer Chromatography

Kiesegel® F60 (Merck, Darmstadt, Germany) was used as the absorbent, and a mixture of cyclohexane, ether, and methanol at the volume ratio 80 : 20 : 1 was used as the mobile phase. 10 mm^3 of 0.50 % solution of each of the samples in 2,2,4-trimethylpentane was applied onto a plate simultaneously with 0.0050 % (w/v) solution of 2-methyl-1,4-naphthoquinone (standard). The dried plate was tested under UV light at 254 nm. The cases where no secondary spot of the samples was more intensive than the spot in the chromatogram of 2-methyl-1,4-naphthoquinone were evaluated as satisfactory.

Sampling

When studying the Ph purification, we used three sets of six samples of materials from three separate batches. The sample *1* of each batch represented the crude Ph after being synthesized, the sample *2* was the distillation residue after degassing the crude Ph (sample *1*). The sample *3* was the distillation residue after distilling off the first fraction from the degassed material (sample *2*) using the first evaporator at 180 ℃ and at a pressure ranging from 0.8 to 2 Pa. The sample *4* was a Ph distillate obtained after degassing and dis-

Table 1. Absorbances of the Phytomenadione Samples at Important Wavelengths Measured in Different Phases of the Purification Process, Ratios of Selected Absorbances, Phytomenadione Content, and Refraction Index
Sample Marking: *1* – crude Ph, *2* – degassed Ph, *3* – Ph after taking the first fraction, *4* – Ph distillate, *5* – Ph distillate after the adsorption, *6* – the first fraction

Batch	Sample	Absorbance							A_{254}/A_{249}	A_{249}/A_{270}	Ph content	n(D, 20 °C)
		0.002 % w/v				0.02 % w/v		100 %				
		243 nm	249 nm	261 nm	270 nm	327 nm	285 nm	550 nm			%	
2910	*1*	0.784	0.820	0.740	0.732	1.36	0.55	> 3.00	0.73	1.12	95.25	1.5560
	2	0.788	0.828	0.748	0.738	1.37	0.55	> 3.00	0.73	1.12	97.80	1.5360
	3	0.789	0.826	0.746	0.736	1.39	0.57	> 3.00	0.74	1.12	98.29	1.5277
	4	0.780	0.820	0.765	0.758	1.35	0.44	1.570	0.72	1.08	98.42	1.5265
	5	0.788	0.831	0.776	0.771	1.36	0.42	0.145	0.72	1.08	100.12	1.5265
	6	0.742	0.773	0.710	0.671	1.23	0.38	2.331	0.75	1.15	93.29	1.5220
3010	*1*	0.797	0.835	0.752	0.738	1.37	0.53	> 3.00	0.74	1.13	95.98	1.5265
	2	0.806	0.846	0.763	0.751	1.39	0.54	> 3.00	0.74	1.13	97.56	1.5270
	3	0.803	0.841	0.759	0.753	1.39	0.55	> 3.00	0.73	1.12	97.93	1.5275
	4	0.775	0.816	0.756	0.753	1.33	0.41	> 3.00	0.72	1.08	99.49	1.5262
	5	0.798	0.838	0.782	0.778	1.36	0.41	0.155	0.72	1.08	99.88	1.5260
	6	0.677	0.701	0.639	0.611	1.11	0.36	2.473	0.74	1.15	80.86	1.5135
3110	*1*	0.771	0.808	0.731	0.722	1.35	0.56	> 3.00	0.70	1.12	97.20	1.5260
	2	0.770	0.807	0.730	0.722	1.38	0.56	> 3.00	0.74	1.12	97.32	1.5260
	3	0.782	0.821	0.740	0.732	1.38	0.58	> 3.00	0.74	1.12	99.64	1.5285
	4	0.773	0.814	0.758	0.752	1.36	0.50	> 3.00	0.73	1.08	99.39	1.5275
	5	0.829	0.873	0.813	0.806	1.43	0.46	0.199	0.73	1.08	99.64	1.5280
	6	0.734	0.760	0.692	0.663	1.20	0.44	0.879	0.74	1.15	89.39	1.5185
Typical values according to												
	BP	0.80	0.84	0.77	0.78	1.4	0.44		0.70—0.75		97.0—102	1.526—1.528
	PBS									1.06—1.10		
	IS							0.200				

A_{254}/A_{249} – A(254 nm)/A(249 nm), A_{249}/A_{270} – A(249 nm)/A(270 nm), Ph – phytomenadione (vitamin K_1), BP – *British Pharmacopoeia* 1993, PBS – *Pharmacopoeia Bohemoslovaca* 1987, IS – internal standard of Slovakofarma, AG, Hlohovec, Slovakia.

tilling off the first fraction (sample *3*) using the second molecular evaporator at 198 °C and at a pressure ranging from 0.3 to 0.6 Pa. The sample *5* was a Ph distillate obtained from the second evaporator (sample *4*) after the adsorption on the DUOLITE® adsorbent, *i.e.* the final product of the vitamin K_1 purification procedure. The sample *6* was the first fraction of the third purification step of the material characterized by the sample *2* obtained by means of the first evaporator at 180 °C.

RESULTS AND DISCUSSION

The purification procedure of processing the crude phytomenadione to a vitamin K_1 having the pharmaceutical quality by means of molecular distillation as the key purification operation consisted of three passages through the molecular evaporators. UV VIS spectral methods and additional analytical tests were used to study the purification of crude Ph. Table 1 contains absorbance values at characteristic wavelengths of UV and VIS regions as well as refraction index values. These data relate to the set of six samples of Ph, which were taken from three batches of the vitamin K_1 manufacturing process at various steps of their purification procedure.

UV absorbances of all the 0.002 % solutions of the samples in various stages of the purification process measured in the region of 243—270 nm were close to the values published in *British Pharmacopoeia* [1]. A significant deviation was found in the first fraction (sample *6*). The closest values to the typical ones were usually found in samples of the product fractions *4* and *5* (Ph distillate and Ph distillate after the adsorption).

The Ph content in the samples from further steps fluctuated within the limits specified by standards, but it was unacceptably low in the initial crude Ph (sample *1*) and, naturally, in the first fraction (sample *6*) where it was substantially lower.

In all the samples, the A(254 nm)/A(249 nm) absorbance ratio was ranging within the typical interval, but in the product fractions (samples *4* and *5*) it was characteristically the lowest.

Only the product fractions (samples *4* and *5*) had the A(249 nm)/A(270 nm) absorbance ratio within the prescribed limits; but this parameter was higher in other samples. However, this parameter is not mentioned by *British Pharmacopoeia* [1], but it is prescribed by *Pharmacopoeia Bohemoslovaca* [6].

The absorbance values of 0.02 % solutions of the samples *1* to *5* in the UV light exhibited no significant differences, notably different values were found in the first fraction (sample *6*). The values of the pure vita-

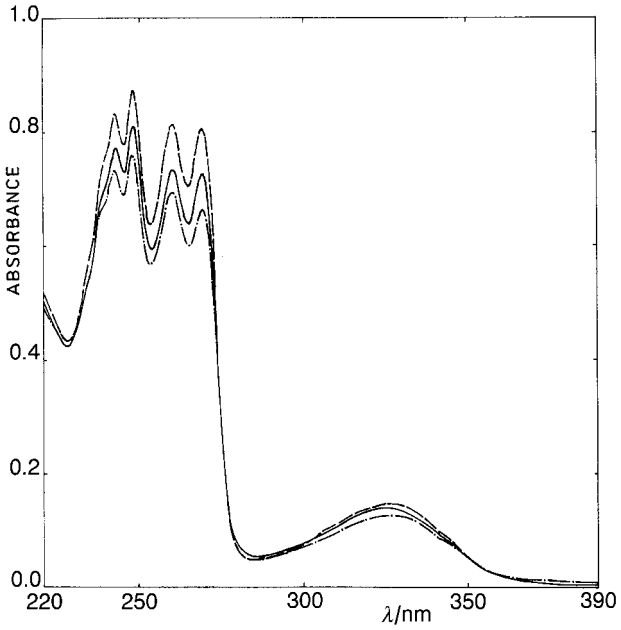

Fig. 1. UV spectra of the solutions of selected samples at various steps of K_1 purification procedure. Batch 3110 from Table 1, sample of a crude Ph (——), of a final Ph after molecular distillation and adsorption (– – –), and of a first fraction (– · – · –) at a composition of 0.002 % (w/v).

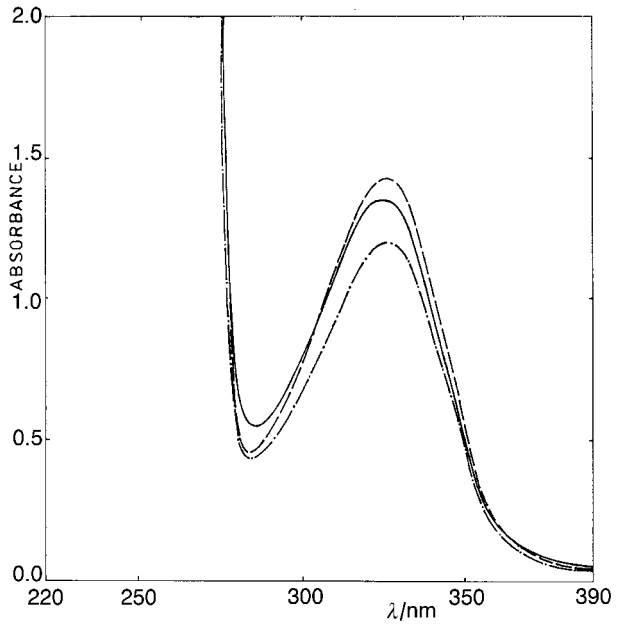

Fig. 2. UV spectra of the solutions of selected samples at various steps of K_1 purification procedure. Batch 3110 from Table 1, sample of a crude Ph (——), of a final Ph after molecular distillation and adsorption (– – –), and of a first fraction (– · – · –) at a composition of 0.02 % (w/v).

min K_1 fractions (samples *4* and *5*) were closest to the standardized data.

In Fig. 1 are shown UV spectra of the solutions of selected samples at various steps of purification procedure. There are presented spectra of the crude Ph, final product, and of the first fraction at a concentration of 0.002 % (w/v). Fig. 2 shows the analogical UV spectra of the same samples at a concentration of 0.02 % (w/v). The differences are illustrative, although not very marked, but decisive are the absorbance ratios.

Significant changes were observed for the absorbance in the visible region at 550 nm where even the distilled Ph sample did not usually meet the required value. This value can be obtained after adsorption using a suitable adsorbent. This purity test is not prescribed by *British Pharmacopoeia*, but it is required by internal specification of Slovakofarma, AG, Hlohovec, Slovakia. Our effort to obtain a satisfactory product only by the adsorption of the treated Ph (after taking the first fraction from the molecular evaporator, sample *3*) was not successful. Only such a procedure, where K_1 was obtained as a distillate, led to a product meeting all the parameters as prescribed.

TLC analysis does not provide sufficient information regarding the extent of product purity requirements because the complete six-sample set of all the batches investigated always complied with the requirements specified in *British Pharmacopoeia* [1], including the sample *6*, *i.e.* the first fraction. Despite that, TLC clearly demonstrates the purification process. In the first three samples, there are spots having their R_F higher than that of the main component. These spots are missing in the further three samples, *i.e.* in the distillates. It is evident that the relevant components remain in the distillation residue. A component with its R_F about 0.46 was found in the chromatograph of the sample *1* (crude Ph). This component concentrates in the sample *6* (first fraction) exclusively and its R_F is equal to that of 2-methyl-1,4-naphthoquinone. The intensity of this component spot in the first fraction was lower than that of the methylnaphthoquinone. No difference between the TLC chromatograms of the samples *4* and *5* (Ph distillate and Ph after adsorption) was observed.

The refraction indices of the first five samples of each batch fluctuated within the interval of typical values, but they were lower in the sample *6*.

The sulfated ash content (experimental values are not given in the table), having the upper limit equal to 0.1 mass %, was ranging from 0.01 to 0.04 mass % in the samples before the distillation and it was equal to zero in the distilled samples (samples *4*—*6*).

CONCLUSION

The performed study on the crude phytomenadione purification by means of molecular distillation using the short-path molecular evaporator showed that this careful distillation method fulfils all the parameters prescribed for a pharmaceutical preparation

and its yield of 85 % is high. The hue of the distillate may be decreased *via* adsorption on a suitable adsorbent.

REFERENCES

1. *British Pharmacopoeia,* pp. 516, 517. Her Majesty's Stationery Office, London, 1993.
2. *Ullmanns Enzyklopädie der technischen Chemie,* 4th Edition, Vol. *23*, p. 653. Verlag Chemie, Weinheim, 1983.
3. Sebrell, W. and Harris, R., *The Vitamins,* Vol. *2*, p. 388. Academic Press, New York, 1954.
4. Kirk—Othmer, *Encyclopedia of Chemical Technology,* 2nd Edition, Vol. *21*, p. 586. Wiley—Interscience, New York, 1983.
5. Cvengroš, J., *Chem. Prum. 40*, 135 (1990).
6. *Pharmacopoeia Bohemoslovaca,* 4th Edition, pp. 747—749. Avicenum, Prague, 1987.