

## PŮVODNÍ PRÁCE

### Preparation and evaluation of hydrophilic cream with propolis extract

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#### SUMMARY

##### Preparation and evaluation of hydrophilic cream with propolis extract

The aim of the study was the preparation and evaluation of a multifunctional hydrophylic cream. The cream was stabilised with a complex emulsifier and carbomer gel, both formed in situ during preparation. The active ingredient was a thick propolis extract in the concentration of 0.8%. The cream with propolis showed antioxidant activity due to the total phenolics content, total flavone and flavonol content. The cream base and the cream with the propolis extract were prepared using various mixing velocities. The properties of preparations studied as response variables were: resistance to centrifugation, viscosity and internal phase particles (droplets) size. The results of examination showed that mixing velocity during preparation process is a significant factor that influences some important characteristics of formulation, mainly rheological properties (viscosity) and particle size, which are closely interlinked and consequently predict the stability of cream preparations during the storage. When the mixing velocity is selected properly, the viscosity of the cream is optimal, and the particle size uniformity as well as the stability of preparation are increased.

**Key words:** hydrophilic cream – propolis – viscosity – particle size – stability

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#### SOUHRN

##### Příprava a hodnocení hydrofilního krému s propolisovým extraktem

Cílem práce bylo příprava a hodnocení hydrofilního krému s širokým spektrem účinku. Krém byl stabilizován komplexním emulgátorem a karbomerovým gelem – oba vznikly in situ během přípravy. Jako účinná složka se použil hustý propolisový extrakt v koncentraci 0,8 %. Krém s propolisem vykazoval antioxidační aktivitu související s celkovým množstvím polyfenolů a celkovým množstvím flavonů a flavonolů. Krémový základ a krém s propolisovým extraktem se připravovaly při různých rychlostech míchání. Jako závislé proměnné se hodnotily tyto vlastnosti přípravků: odolnost k centrifugaci, viskozita a velikost částic (kapek) vnitřní fáze. Výsledky hodnocení ukázaly, že rychlost míchání během procesu přípravy patří mezi významné faktory, ovlivňující některé důležité charakteristiky přípravku, především reologické vlastnosti a velikost částic, které jsou vzájemně propojené, a následně

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předpovídají stabilitu krémových přípravků během uchovávání. Pokud rychlost míchání je určena správně, má krém optimální viskozitu a uniformita částic stejně jako jeho stabilita vzrůstá.

**Klíčová slova:** hydrofilní krém – propolis – viskozita – velikost částic – stabilita

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Má

## Introduction

Propolis (bee glue) is a resinous substance that bees collect from leaves, buds and damaged sites of a large variety of trees (birches, poplars, pines, alders, willows). The collected substance is enriched by bee salivary secretions and enzymes. Chemical composition of propolis is highly variable – it is composed of more than 200 components including phenolic acids and their esters, flavonoids, caffeic acid and esters, ferulic acid and others<sup>1–4)</sup>. The chemical profile of poplar propolis, the best known type of bee glue, can be characterized by the following three parameters: total flavone and flavanol content, total flavanone and dihydroflavonol content, and total phenolics content<sup>5, 6)</sup>. Because of the presence of biologically active compounds, propolis exhibits antimicrobial<sup>1, 7, 8)</sup>, antifungal<sup>9)</sup>, antiviral<sup>10)</sup>, antioxidant effects<sup>1, 8, 11, 12)</sup>, alleviates pain and reduces inflammation<sup>1, 8, 13, 14)</sup>, promotes healing of wounds and ulcers<sup>15)</sup>, and activates the processes of regeneration<sup>16)</sup>. That is why propolis is used topically in the form of creams, ointments, gels, lotions or solutions<sup>17–19)</sup>.

Creams are semisolid emulsion systems having a creamy appearance as the result of reflection of light from their emulsified phases<sup>20)</sup>. Hydrophilic creams are o/w emulsions with a hydrophilic external phase, usually water. They are miscible with water and skin secretions and thus they are easily removed from the skin or clothing<sup>21)</sup>. They are not occlusive because they leave little residue on the skin. Properly designed o/w creams are elegant systems, pleasing in both appearance and feel post application<sup>20)</sup>. They are good for most topical purposes and are quite acceptable from a cosmetic viewpoint.

Creams, as multiphase preparations – the base consists at least of two immiscible components (usually called oil and water) – require to be stabilised. It is possible to form emulsion systems that are kinetically stable for a reasonable period of time by including substances known as emulsifiers and/or thickening agents<sup>22)</sup>. From the stability standpoint, the addition of an emulsifying agent is critical to formulating an emulsion<sup>21)</sup>. Hydrophilic creams are usually prepared with more than one emulsifying agent (emulsifier mixtures), resulting in an HLB of about 8 to 16<sup>21)</sup>. Some of o/w creams contain stearic acid, and then triethanolamine stearate, which is usually prepared *in situ* during manufacture of cream, is one of the quite popular emulsifiers for creams and lotions today in combination with w/o emulsifying agents, e.g. waxy alcohols, long-chain esters etc.<sup>20, 21)</sup>. Various self-emulsifying bases containing the mixture of

emulsifying agents (o/w and w/o) may be used for these purposes as well<sup>23, 24)</sup>. Thickening agents are used to increase viscosity of the continuous (external) phase of emulsions, and they enhance emulsion stability by retarding the movement of droplets (particles of internal phase)<sup>22)</sup>. Moreover, manufacture procedures such as the energy of mixing and hence the level of dispersion may considerably influence the characteristics and the stability of the cream system<sup>25, 26)</sup>. Propolis may be incorporated into the cream formulation in the form of a thick or liquid ethanolic or glycolic extract<sup>23, 24)</sup>.

The aim of the study was the preparation and evaluation of a multifunctional hydrophilic cream with a propolis extract.

## EXPERIMENTAL PART

### Chemicals and excipients

The chemicals and samples were available commercially and were used as received: gallic acid, Folin-Ciocalteu reagent (Fluka), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin (Sigma). All other chemicals used were of analytical grade. Pharmaceutical excipients used for the preparation of a cream such as mineral oil (Paraffinum liquidum), white petrolatum (Vaselinum album), stearic acid (Acidum stearicum), glycerol monostearate (Glyceroli monostearas), carbomer 934P (Carbomerum), triethanolamine (Trolaminum) and purified water (Aqua purificata) were of Ph. Eur. quality.

### Preparation of cream base and cream with propolis extract

The composition of the cream base and the cream with a thick propolis extract (AB “Valentis”, Lithuania) is presented in Table 1.

Hand-made creams were prepared in a mortar with a pestle. Parts A and B were heated separately to 70 °C. Part C compound carbomer was previously spread in a thin layer on the water surface, kept for 8 hours until it was dissolved, and subsequently heated to 70 °C. Part A was added to Part B with stirring, and Part C was added afterwards. The mixture was continuously stirred until the temperature of 35 °C was reached. Then Part D was added, mixed properly, and the cream was cooled with stirring to room temperature.

Table 1. Composition of cream formulations

Part	Compound	Formulation (% weight)	
		Cream base	Cream with propolis extract
A	Vaselineum album	12.0	12.0
	Paraffinum liquidum	12.0	12.0
	Glyceroli monostearas	4.3	4.3
	Acidum stearicum	5.0	5.0
B	Trolaminum	2.0	2.0
	Aqua purificata	30.0	30.0
C	Carbomerum	0.2	0.2
	Aqua purificata	34.5	32.9
D	Propolis extract	–	0.8
	Ethanolum 70% (V/V)	–	0.8

For the preparation of other creams, a Pilmixer mixer (Danifoss, Denmark) was used. Parts A, B and C were prepared as presented above. Mixing in the same sequence and primary emulsification at 70 °C and the mixing velocity of 200 rpm followed after that. While being stirred continuously (200 rpm), the emulsion was cooled to 60 °C, and then homogenised at variable speeds (4000, 5000, 7000 or 9000 rpm). After homogenisation the batch was cooled to 35 °C at a rate of 100 rpm. After addition of Part D, cooling and stirring continued until room temperature was reached.

#### Preparation of cream extracts for further evaluation

Creams were extracted 6 times by obtaining 0.50 g of the product and 10 ml of the extractant, a 96% (V/V) ethanol and isopropanol mixture at the ratio of 8:2 (V/V). The resulting extracts were mixed, and the extractant was added to reach a 100 ml volume. Three such extracts were produced from the cream base and the propolis cream.

#### Total polyphenol content

Total polyphenol content was determined using the colorimetric method. 2.0 ml of the prepared extract was oxidized using Folin-Ciocalteu reagent (400 µl), and a sodium carbonate solution (75 g/l) was then added to the reaction mixture to reach a 10.0 ml volume. After 2 hours, the suspension was centrifuged for 10 minutes at 5000 rpm, and absorption was measured at a 760 nm wavelength. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 g of the sample (extract).

#### Total flavone and flavonol content

Flavones and flavonols contents were analyzed by the

colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 minutes, absorption was measured at 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg per 100 g of the sample (extract).

#### DPPH reduction

The free radical scavenging ability was investigated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The prepared extract (400 µl) was replenished up to 2.0 ml with 0.1 mM of DPPH methanol solution, and absorption was measured after 30 minutes at a 517 nm wavelength. DPPH reduction was calculated when taking into account the absorption of the control investigation, and the observed activity was compared to the quercetin calibration curve. The results were expressed as quercetin antioxidant activity equivalent (QE) µmol per 100 ml of the solution (extract).

#### Microscopic examination of the cream

The particle size of the internal phase was measured using an optical microscope (Lambda, Ltd.), which was fitted with a camera and computer software (LECO Corp., Michigan, USA) for image analysis transmitted on the monitor. The microscopic samples were prepared by spreading a very thin layer of the cream on the specimen slide and pressing it well with a cover slip. Three preparations were made by taking small amounts of the cream from different places. The preparations were observed under passing light. As the first step, the preparations were inspected under a 100-fold magnification, and measurements were performed under a 400-fold magnification. In each of the three preparations, 100 particles were measured. In addition to that, photographs of the preparations were made during microscopy. On the basis of measurements the degree of dispersity was calculated.

#### Differential centrifugation

The resistance of the formulated creams to centrifugation was tested in a 20 ml-graduated cylinder at 10,000 rpm for 1–20 min at different temperature conditions, such as 4 °C, 25 °C, and 40 °C, using a centrifuge Ependorf 5810-R (Sigma, Germany) as described by Cockton<sup>27)</sup>.

#### Organoleptic evaluation

The organoleptic features of the cream were examined at the same temperature (25 ± 2 °C), lighting and humidity (60% RH) conditions to assess variations in appearance, phase separation, colour, or smell. Samples were evaluated after 1, 7, 14, 30 days of preparation, then each month up to the end of the 14<sup>th</sup> month after preparation.

### Viscosity measurement

The viscosity of each sample was measured by a rotational viscometer Selekt P (Abrera, Spain) at various speeds (rpm): 1, 2, 5, 10, 20, 30, 60, 100, 200 at  $25 \pm 0.1$  °C, using a spindle R7.

### Statistics

Data were presented as means  $\pm$  S.E.M. The nonparametric methods were applied for making inferences about the data. Differences between the mean values in dependent groups were tested using the Wilcoxon matched pairs test. Differences between the mean values in independent groups were tested using the nonparametric Kruskal-Wallis test with the Dunns post-hoc evaluation.  $P < 0.05$  was taken as the level of significance. Statistical analysis was performed by using the software package Statistica 1999, 5.5 StatSoft Inc., USA.

## RESULTS AND DISCUSSION

At the beginning of the study, a cream base and a cream with the propolis extract were prepared. Taking in consideration the composition and preparation technology, the product was believed to be of the o/w emulsion type, i.e., a hydrocream stabilized by a complex emulsifier: primary – trolamine stearate (o/w) formed during the process of preparation, and secondary

– glycerol monostearate (w/o) contained in the lipophilic phase. Emulsions with a complex emulsifier, according to the data from the literature <sup>21, 28</sup>, have a better quality than those stabilised with an o/w type emulsifier only. The test with colouring agents (Sudan III, methylene blue) and the electrical conductivity test confirmed the development of a hydrocream. The cream was additionally stabilised by increasing viscosity of the external phase emulsion with carbomer gel formed *in situ*.

Antioxidant activity of the cream with propolis was evaluated by measuring the stable DPPH. The cream with propolis showed antiradical activity due to the total phenolics content, total flavone and flavonol content (Table 2). These parameters correlate better with the biological activity and are more informative than the quantification of individual components <sup>5</sup>. Our results correspond to the findings of Marquele et al. <sup>24, 28</sup>, reporting that topical formulations with a liquid propolis extract exhibit considerable antioxidant activity. Thus, the development of topical formulations with propolis is justified, because such preparations may prevent and treat skin damages caused by free radicals generated in the aging process and by external stimuli such as sunlight <sup>28</sup>.

The outcomes of rheological studies showed that rheological characteristics of creams depend statistically significantly on the mixing velocity during preparation (Table 3). When the hand mixing was used, the cream had the least viscosity. When rpm of the viscometer was 1.0, the viscosity of the hand-made cream was 1.5-fold lower ( $p < 0.05$ ), compared with the viscosity of the cream prepared at 4000 rpm. The further studies showed

Table 2. Antioxidant activity of hydrophilic creams

Formulation	Total polyphenol content (GAE mg/100 g) <sup>a</sup>	Total flavone and flavonol content (QE mg/100 g) <sup>b</sup>	DPPH reduction (QE $\mu$ mol/100 g) <sup>c</sup>
Cream with propolis extract	66 $\pm$ 8	2.62 $\pm$ 0.72	132 $\pm$ 23
Cream base	< 50	< 1	< 50

<sup>a</sup> – data presented as gallic acid equivalent (GAE) mg per 100g of the extract

<sup>b</sup> – data presented as quercetin equivalent (QE) mg per 100g of the extract

<sup>c</sup> – data presented as quercetin equivalent (QE) micromoles per 100g of the extract

Table 3. The rheological properties of propolis cream in dependence on various mixing velocities

Mixing velocity during preparation (rpm)	Viscosity of preparations (mPa . s) at various rpm of viscometer								
	1.0	2.0	5.0	10.0	20.0	30.0	60.0	100.0	200.0
hand mixing	40867 $\pm$ 578	34998 $\pm$ 475	23869 $\pm$ 124	15854 $\pm$ 145	9862 $\pm$ 83	7592 $\pm$ 187	4744 $\pm$ 147	3438 $\pm$ 78	2366 $\pm$ 15
4000	62140 $\pm$ 1270	52101 $\pm$ 879	47891 $\pm$ 549	32181 $\pm$ 254	21472 $\pm$ 143	12852 $\pm$ 149	7542 $\pm$ 247	6459 $\pm$ 136	3214 $\pm$ 118
5000	71451 $\pm$ 1104	62541 $\pm$ 1147	54962 $\pm$ 997	40130 $\pm$ 683	20196 $\pm$ 254	14568 $\pm$ 547	8265 $\pm$ 278	6354 $\pm$ 168	3897 $\pm$ 132
7000	97241 $\pm$ 879	81569 $\pm$ 968	70987 $\pm$ 845	46981 $\pm$ 368	27184 $\pm$ 487	20998 $\pm$ 194	11687 $\pm$ 127	8141 $\pm$ 149	4989 $\pm$ 165
9000	99432 $\pm$ 547	81456 $\pm$ 657	71647 $\pm$ 758	47712 $\pm$ 254	27292 $\pm$ 136	20906 $\pm$ 450	11882 $\pm$ 187	8291 $\pm$ 215	5003 $\pm$ 165



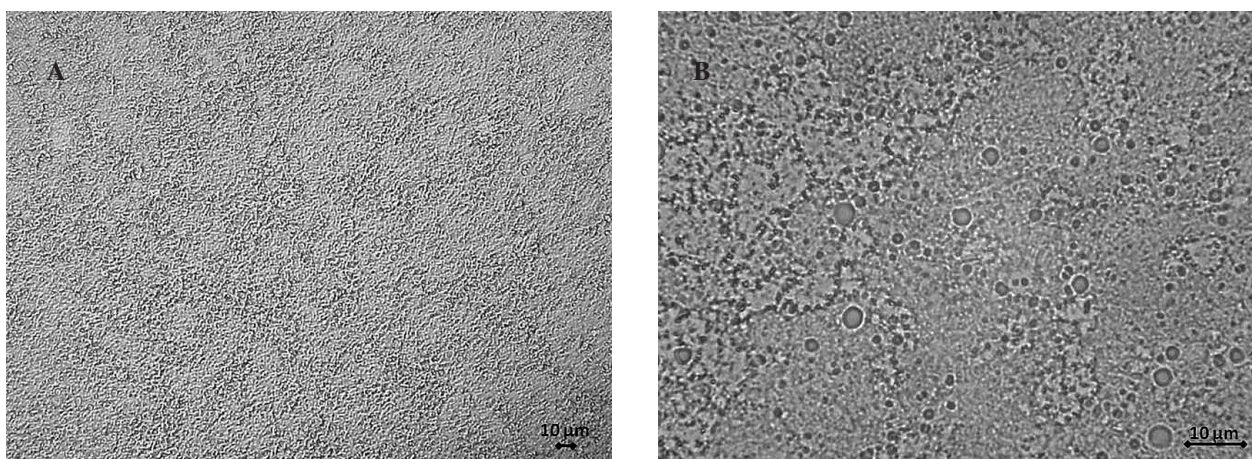


Fig. 1. Microscopic image of the propolis cream at a 100-fold magnification (A) and a 400-fold magnification (B)

that with an increase in the mixing velocity, the viscosity of the cream also increases up to 7000 rpm. The viscosity of the cream prepared at 9000 rpm did not differ significantly from that prepared at 7000 rpm. An addition of 0.8% of a thick propolis extract did not influence the viscosity of the cream base (data not shown).

Particle (droplet) size and particle size distribution are very important characteristics of the emulsion system, because they indicate the quality and stability of the preparation as well as the effectiveness of the manufacturing process and the influence of the emulsifier on the quality of the emulsion<sup>22, 24)</sup>. If the active substance is in the internal phase, the particle size influences bioavailability. Consequently, it is important to be able to reliably control, predict, measure, and report the size of the droplets in emulsions<sup>22)</sup>. In our study, microscopic evaluations of creams prepared at different mixing velocities were made. Particle size and particle size distribution (degree of dispersity) depended markedly on the mixing procedure. The degree of dispersity was lower in the case of the cream base prepared at lower velocities and varied from  $0.9 \times 10^{-4} \text{ cm}^{-1}$  (hand mixing) to  $1.16 \times 10^{-4} \text{ cm}^{-1}$  (5000 rpm). When the mixing velocities were 7000 rpm and 9000 rpm, a uniform particle size distribution was observed. For further microscopic evaluation, the cream base and cream with propolis prepared at 7000 rpm were used. The images of these preparations are presented in photographs (Fig. 1). When the preparation was magnified 100-fold, a very homogenous, uniform picture was seen. At a 400-fold magnification, the majority of particles were very small, a few larger particles and no particles greater than 10 µm were found.

According to the data from the literature, the diameters of particles in most emulsion preparations usually lie somewhere between 0.1 and 100 µm<sup>22)</sup>. Stokes's law states that the velocity at which a droplet moves is proportional to the square of its radius. The stability of an emulsion can therefore be enhanced by reducing the size of the droplets. In general, both small oil droplets (lipophilic particle in o/w cream) and high viscosity contribute to the stability of the emulsion<sup>29)</sup>.

Conventional emulsion preparations differ as

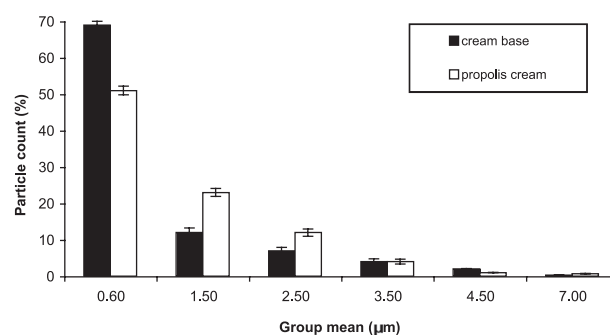


Fig. 2. A comparison of the the internal phase particle size in the cream base and the propolis cream

polydisperse, i.e. they always contain a distribution of droplet sizes<sup>22)</sup>. Therefore a knowledge of the average size of the particles and the width of the distribution is often sufficient. The number of droplets (particles of the internal phase) in most emulsion preparations is extremely large, and so their size can be considered to vary continuously from some minimum value to some maximum value. The particles size of our preparations varied from 0.131 µm to 7.352 µm with the mean diameter of 1.13 µm (cream base) or from 0.13 µm to 6.833 µm with the mean diameter of 1.352 µm (propolis cream). Just these results predict the good quality of prepared creams. However, when presenting the particle size data, it is convenient to divide this size range into a number of discrete size classes and the number of droplets that fall into each class<sup>22)</sup>. The resulting data can then be represented in a tabular form or plotted as a histogram that shows the number of droplets in each size class. In our case, all measured particles were divided into 7 classes (Fig. 2). As the graph illustrates, the major part of cream particles falls into the first class with the group mean of 0.60 µm, which confirms a high quality of prepared hydrocreams. The results of a comparison of the cream base with the propolis cream suggested that the difference of droplet number in the class between the cream base and the cream with propolis extract was slight (Fig. 2), i.e., in the propolis cream there were fewer particles up to 1 µm than in the cream base (55.33% vs. 69.67%). However, the total

number of particles up to 3  $\mu\text{m}$  did not differ (92.00% vs. 92.67%). The degree of dispersity was  $1.64 \times 10^{-4} \text{ cm}^{-1}$  and  $1.69 \times 10^{-4} \text{ cm}^{-1}$ , respectively. The obtained results show that both the cream base and propolis cream belong to stable emulsion systems.

The stability of the creams was also evaluated by centrifugation for 1–20 minutes at 4 °C. The formulations that were resistant were selected for further evaluation at higher centrifugation temperatures of 25 °C and 40 °C. The creams with propolis manufactured by hand mixing and at 4000 rpm lost stability in 7–10 minutes. The cream manufactured at 5000 rpm remained stable at 4 °C temperature for 20 min. However, by increasing temperature up to 25 °C the stability was maintained for 10 min and at 40 °C creams held the line only 2 minutes, then the separation of the water and oil phases was observed. The creams with propolis manufactured at 7000 rpm and 9000 rpm remained resistant to centrifugation for 20 min even at 40 °C temperature. The centrifugation test is of major interest since it may provide fast information about probable behaviour of emulsion preparations during storage. In our experiment, the creams resistant to centrifugation at the most exacting conditions remained stable for all the time of investigation.

Organoleptic evaluation of creams was maintained for 14 months. Immediately after preparation all formulations were homogenous. The cream base was white and glossy. An addition of the propolis extracts to the preparations resulted in the formulations with characteristic appearance, colour and smell. Stability of the creams manufactured at the different mixing velocities differed: the creams produced at a higher rotation number per min were more stable for a longer period. Namely, the hand-mixed cream with the propolis extract maintained its stability for 4 months, the cream manufactured at 4000 rpm, for 6 months, at 5000 rpm, 10 months, 7000 and 9000 rpm, 14 months. Instability appeared as the changes in colour, surface appearance or phase separation.

In conclusion, mixing velocity during the process of preparation is a significant factor that influences some important characteristics of formulation, such as rheological properties (viscosity) and particle size, which are closely interlinked and consequently predict the stability of cream preparations during the storage. When the mixing velocity is selected properly, the viscosity of the cream is optimal, and the particle size uniformity as well as stability of preparation increases.

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## PRAKTICKÁ LÉČBA DIABETU

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Přestože předkládané postupy vycházejí z platných standardů léčby diabetu, existuje v řadě případů i postup alternativní. Pokud tomu tak je, snažili se autoři tuto možnost v rámci knihy zmínit a zároveň vysvětlit, v čem považují jimi doporučený postup za vhodnější.

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