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Physical and chemical properties of emulsions made of ingredients obtained from Juglans regia L.

KATARZYNA WOJCIECHOWSKA, MARIA ZUŃ, DOROTA DWORNICKA, SYLWIA WOŚKO, EWA POLESZAK

* Chair and Department of Applied Pharmacy, Medical University of Lublin, Poland

ABSTRACT

The aim of the study was to obtain emulsions containing walnut oil, defatted, powdered walnut seeds and water. The walnut oil and defatted walnut seeds were obtained by extraction method using chloroform and methanol in proportion (1:1), (v/v). The oil was investigated according to Polish Pharmacopea VIII (organoleptic value, density, oil number and saponoating number). The defatted seeds were analyzed organoleptic and granulometric analysis of the particle size was done. Nine different emulsions were prepared and next physical and chemical properties of these formulas were investigated.

Keywords: emulsion, emulsifier, rheology, Juglans regia L.

INTRODUCTION

Emulsion are heterogeneous dispersed formulas made from two immiscible liquids phase substances dispersed one into each other [17]. As a hydrophilic phase water, glycerol, ethanol can be used; [13, 17], and as a lipophilic phase mineral oil [15], plant oil like almond oil [2], macadamia nut oil [3], oat oil [10], olive oil [22] and walnut oil [28] can be used.

In emulsions it is necessary to use an emulsifier. The synthetic substances like natrii laurylosulfate [1, 16, 17], Span, Tween are usually used [6]. There are popular biopolymers like mannan and glucomannam obtained from yeast, which are recently used as natural emulsifiers [14].

Emulsions can be divided into two groups. Emulsion in which the lipofilic phase is dispersed in the aqueous continued phase is called oil in water (O/W), and emulsion in which the water is dispersed into oil phase is called water in oil (W/O) [17].

Nowadays there is a great interest in making semisolid formulations for dermatological use, which have nursing, moisturizing and protective properties.

In the current literature there is a lot of information about using plant oil for preparation of emulsions but there is not enough information about using walnut oil for making dermatological preparations. There is also no information about using post extraction components obtained from walnut seeds as a natural gel agent and partially self-emulsifying source.

Walnut (Juglans regia L.) is a member of Juglandaceae family and it is native in Southeastern Europe, Asia Minor, India and China [19, 28].

Walnut oil shows beneficial action on the skin. Walnut oil contains 10% saturated fatty acids (palmitic and stearic acid), and 90% unsaturated fatty acids from which 9-30% is oleic acid, 57-76% is linolic acid and 2-16% is linolenic acid [19]. Because of high content of essential fatty acids, especially polyunsaturated fatty acids (PUFA), walnut oil can be used as a very important ingredient for cosmetic's creams. PUFA have nursing, moisturizing and protective properties. They act between the skin lipids and they reduce trans-epidermal water loss [18, 24].

Walnut seeds are rich source of vitamin E. The highest amount of tocopherol is beta and gamma tocopherol 19.5 mg/100g, followed by delta tocopherol 3.05 mg/100g and alfa tocopherol 2.6 mg/100g [9]. Vitamin E has antitumorigenic, photoprotective and skin barrier stabilizing properties [23].

To separate lipophilic compounds like lipids, phospholipids and tocopheroles, non polar solvents as methanol, acetone, chloroform, acetonitrile (or their mixture) are used [4, 5, 8].

Corresponding author
* Chair and Department of Applied Pharmacy,
Medical University of Lublin, Chodźki 1 Str., 20-093 Lublin
e-mail: kgierucka@wp.pl

Physical and chemical properties of emulsion depend on emulsifier used water and oil contain and methods of homogenization [17, 20, 21].

Realdon [27, 28] investigated emulsions where for homogenization turbo mixer and blender were used. When the speed rates of turbo mixer or blender increases the viscosity of the emulsions will increase too [27].

The most popular investigations in emulsion formulas are determinations of saturated emulsion type, particle size of internal phase, density and rheological properties.

Rheological properties of emulsion are the most important physical parameters in manufacturing, filling, storage and esthetic look. Those investigations determine quality and usefulness of emulsions [9].

Based on the collected information semi-solid emulsions for dermatological use were prepared.

Those emulsions contained walnut oil, water and defatted, powdered walnut seeds. Different amounts of walnut oil and water were mixed. As a gelling agent and partially self-emulsifying source, defatted walnut seeds were added.

Next physical and chemical properties of these emulsions were investigated.

MATERIALS AND METHODS

The seeds of Juglans regia were collected in South of Poland in 2008. Used solvents: chloroform, methanol (purchased in POCH, Gliwice).

Raw material treatment and method of extraction

The seeds of Juglans regia were collected in the South of Poland in 2008.

The walnuts were stored till the day of experiment in the temperature of 10°C. On the day of the experiment the walnut were striped from husk, crushed and sieved. After that, 1000 g of walnut were placed in percolator, wetted with mixture of solvent chloroform and methanol 1:1 (v/v) [8]. Walnut has stayed in percolator for 24 h and on the next day percolation process was performed. The process was repeated three times. Such composition of solvents and the way of extraction seemed to be the most optimal and it enhanced full extraction of lipophilic and hydrophilic substances from the walnut kernel.

After that, the solvents were distillated from the liquid extract in the rotary evaporator at lowered pressure and the oil was separated from the mixture.

Defatted walnut seeds were dried and powdered.

Characteristics of walnut oil

Characteristics of walnut oil were investigated. Investigation included evaluations of density, acid number and saponating number according to Polish Pharmacopea VIII.

Results:

- Oil was clear with yellow color and characteristic walnut smell.
- Density of the oil measured by the pycnometer was 0.875 g/ml and it was lower than presented in literature – 0.945-0.970 g/ml [19, 26].
- Acid number was 0.397 mg KOH/ 1g of the oil.
- Saponating number was 296.2 mg KOH/ 1g of the oil and it was higher than presented in literature – 161.4 mg KOH/ 1g of the oil [19].

Characteristics of defatted walnut seeds powder.

Based on the organoleptic and granulometric analysis of the obtained powder it was determined that:

- Powder was semi yellow, without smell.
- Powder was not soluble in water and other solvents like methanol, ethanol, and chloroform and expanded when it was mixed with water.
- Sizes of powder particulates, measured by the projector microscope (Lanametr) were between 1.25 μm to 12.5 μm and the average size was 6.62 μm.

Formulas and emulsions preparation

From defatted and powdered walnut seeds, water and walnut oil nine emulsions R1a –R9a were prepared. Those emulsions have the same quantity of defatted, powdered walnut seeds and different proportions of water and oil. The amount of water and oil in those emulsions is shown in table 1.

Table 1. Amount of water and oil in emulsions R1a – R9a

Emulsion	The amount of defatted walnut seeds (g)	Oil (g)	Water (g)
R1a	1.5	10	10
R2a	1.5	9	11
R3a	1.5	8	12
R4a	1.5	7	13
R5a	1.5	6	14
R6a	1.5	5	15
R7a	1.5	4	16
R8a	1.5	3	17
R9a	1.5	2	18

To obtain emulsions the defatted, powdered walnut seeds were placed in the mortar and the appropriate amount of water was added. The mixtures have been triturated for 30 minutes. After that time the oil was added and the triturating have been continued. At the end, the emulsions have been homogenized for 5 min. in the turbo mixer at the speed rate 10 000 / min.

PHYSICAL AND CHEMICAL TESTING OF THE OBTAINED EMULSIONS

Organoleptic evaluation

All emulsions had the consistency of cream with brown color and walnut smell. They were homogenous, non-greasy, and smooth in texture and they didn't form a greasy film on the skin upon application.

Determination of emulsion type

Based on the indicative method presented in the Polish Pharmacopeia VI and observation under microscope, it was determined that those emulsions are oil in water type.

Determination droplet size of internal phase

Particle sizes of the oil phase in emulsions were determined using projection microscope (Lanameter). The diameter of 300 particles were determined and average (median) of the diameter was calculated according to the method described by Burlage [5].

It was also determined that when the amount of water in emulsion increases the particle size will decrease.

Determination of density of the emulsions

Density of those emulsions was carried out accordingly to FP VIII, by the use of picnometer. Each time when picnometer was tarred, it was filled with an appropriate emulsion, and it was weighed.

Density of emulsion will increase when the water amounts increases too.

Particle size and density of each emulsion is shown in table 2.

Table 2. Particle size and density of emulsions R1a–R9a

Emulsion	Contain of water (%)	Average oil droplet size (μm)	Density (g/ml)
R1a	46.51	36.03	0.945
R2a	51.62	34.51	1.031
R3a	55.81	34.30	1.036
R4a	60.47	25.38	1.044
R5a	65.12	25.98	1.056
R6a	69.77	24.96	1.040
R7a	74.42	19.05	1.090
R8a	79.07	15.93	1.084
R9a	83.72	12,65	1.092

Rheological properties of the emulsions

The flow characteristic is the most important property of a semi-solid emulsions because it determines its quality, usefulness and purpose [17, 18].

Rheological properties of the emulsions were determined by using „Rheotest -2" Medingen viscosity meter and cylinder set S/S1, at the increasing and decreasing rotation speed (Dr) in the range of 1.5 s^{-1}-656 s^{-1}. The values α were read on the scale. The value of the shear stress Tr was calculated by the following equation:

$$Tr=Z\alpha$$

where:
Tr – shear stress (10^{-1} Pa)
Z – cylinder constant (10^{-1} Pa/Skt)
α – the values on the scale (Skt)

The values of shear stress at the increasing and decreasing shear rates (Dr) are shown in Fig. 1.

Examined emulsions have pseudoplastic character of flow, but when the water content increases the pseudoplastic character of flow conformed to Newtonian flow. Moreover the values of shear stress (Tr) measured at increasing and decreasing shear rates (Dr) show that there are no thixotropy properties in examined emulsions.

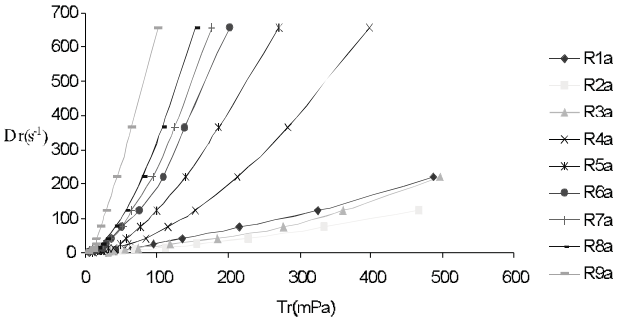

Fig. 1. The values of shear stress (Tr) and shear rates (Dr) for the emulsions R1a to R9a

Viscosity of the emulsions is dependent on the water content. The viscosity of the emulsions will decrease when the water content increases.

Based on the Fig. 2 the areas under curves ($AU_\eta C$) were calculated by the following equation:

$$AU_\eta C = \Sigma[(\eta_{(i-1)}+\eta_i)/2]x(Dr_i-Dr_{(i-1)}).$$

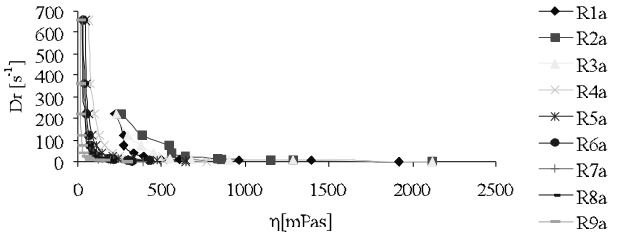

Fig. 2. Changes of viscosity (η) of emulsions R1a – R9a according to the share rate (Dr)

Then the natural logarithms of the values were calculated. The values of $AU_\eta C$ and $lnAU_\eta C$ of investigated emulsions are shown in table 3.

Table 3. The $AU_\eta C$ and $lnAU_\eta C$ values of the emulsions R1a-R9

Emulsion	Contain of water (%)	$AU_\eta C$ (mPa)	$lnAU_\eta C$
R1a	46.51	110225.85	11.61
R2a	51.62	139830.98	11.85
R3a	55.81	131947.17	11.79
R4a	60.47	68444.43	11.34
R5a	65.12	47511.00	10.77
R6a	69.77	34109.74	10.74
R7a	74.42	29921.65	10.31
R8a	79.07	25798.23	10.16
R9a	83.72	15805.12	9.67

The influence of the water content on the values of lnAU?C was presented in Fig. 3.

The values of $lnAU_\eta C$ decrease linearly in the emulsions when the water content is between 46.51-83.72%. The linear character of the figure can be described by the equation.

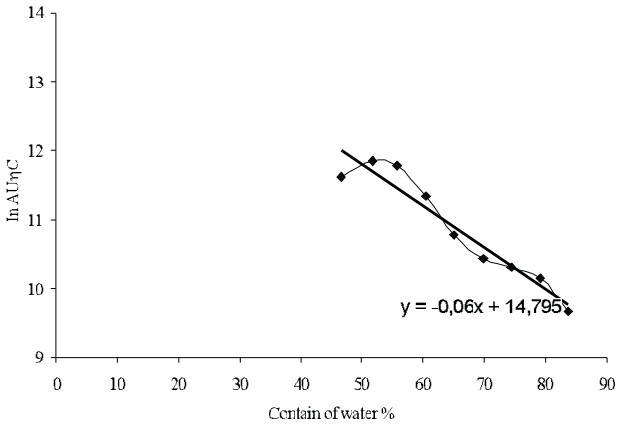

Fig. 3. The influence of the water content (%) on the values $lnAU_{\eta}C$ for emulsions R1a-R9a

$$lnAU_{\eta}C = \ln AU_{\eta}C_0 - KxW$$

where;
W – contain of water %
$lnAU_{\eta}C_0$ – 14.795
K – 0.06

Thus, the final equation is:

$$\ln AU_{\eta}C = 14.795–0.06W$$

Based on the equation, the value of $AU_{\eta}C$ for different amount of water in emulsion can be described.

DISCUSSION

Emulsions contained walnut oil, defatted and powdered walnut seeds and water. This type of the emulsion was stable in room temperature during the period of investigation.

They had creamy consistency, brownish color, walnut smell and they were like vanishing creams because when they were applied on the skin of hand they formed a greasy film which stayed only less than one hour.

Droplets size of the internal (oil) phase of these emulsions decreases from the 36.03 μm (in diameter) to 12.65 μm with increase of the water amount from 46.51% to 83.72%.

Density of these emulsions increases from the 0.945 g/ml to 1.092 g/ml when the water amount in the emulsions increases from 46.51% to 83.72%.

All emulsions showed pseudoplastic flow without a yield point. Those emulsions didn't show thixotropic properties. When the water content increases the flow is similar to Newtonian flow. When the share rate is 1.5 s^{-1} the viscosity of the emulsions decreases from the 1923.33 mPas for the R1a formula to the 288.50 mPas for the R9a formula.

Till the day there has been no publication about these emulsions in which the main ingredients are: defatted walnut seeds as natural emulsifying agent, walnut oil and water.

Investigated emulsions had very similar properties like other emulsions described in literature, which contain natural or synthetic emulsifier.

Obtained emulsions can be used in future as a drug carrier but more investigations are necessary.

CONCLUSIONS

1. Micronized powder of defatted walnut seeds introduced into the system of two immiscible liquids water and oil acts as untypical emulsifier.
2. The emulsifying action of the powder remained in the system walnut oil : water mixed in the proportion 1:1 to 1:9.
3. Obtained preparations were oil in water type and the droplet size of internal phase was 12.65-36.03 μm.
4. In rheological studies conducted at room temperature all the preparation had pseudoplastic flow and their viscosity decreased with the increase of water in the emulsions.

REFERENCES

1. Achim M. et al.: Topical dosage forms containing fluidextract of ruscus aculeatus: Formulation, preparation and physical characterization. *Clujul Medical.,* 83, 99, 2010.
2. Akhtar N. et al.: Formulation and in vitro evaluation of a cosmetic emulsion from almond oil. *Pak. J. Pharm. Sci.*, 21, 430, 2008.
3. Akhtar N., Yazan Y.: Formulation and in vivo evaluation of cosmetic multiple emulsion containing vitamin C and wheat protein. *Pak. J. Pharm. Sci.,* 21, 2008.
4. Amaral J. S., et al.: Triacyloglycerol composition of walnut (Juglans regia L.) cultivars: Characterization by HPLC-ELSD and chemometrics. *J. Agric. Food Chem.*, 52, 7964, 2004.
5. Bao – Yuan G., et al.: Separation and determination of phospholipids in plant seeds by nonhydrous capillary electrophoresis. *J. Chromatogr A.*, 205, 1074, 2005.
6. Broniarz-Press L., Sadowska J.: *Wpływ ozonowania i surfaktantów na reologię kosmetycznych emulsji o/w.* XIX Ogólnopolska Konferencja Inżynierii Chemicznej i Procesowej. Rzeszów 2007.
7. Burlage H. M., Charles O. Lee, L.: *Physical and technical pharmacy*. The McGraw-Hill Book Company, 1963.
8. Czarnecki W., Gierucka (Wojciechowska) K.: *Maść do pielęgnacji skóry na bazie wyciągu z nasion orzecha włoskiego oraz sposób jej otrzymywania.* Patent nr 204335, Warszawa 2010.
9. Delgado M.M., Bustamente R., Sanchez-Perez A.: Pressurized liquid extraction prior to liquid chromatograpy with electrochemical detection for analysis of vitamin E isomers in seeds and nuts. *J. Chromatogr A.*, 249, 1056, 2004.
10. Dull BJ.: Oat oil for personal care products. *C&T.*, 112, 77, 1997.
11. *Farmakopea Polska VI,* PZWL, 2002.
12. *Farmakopea Polska VIII*, PZWL, 2008.
13. Gloor M., Bettinger J., Gehring W.: Modification of stratum corneum quality by glycerin-containing external ointments. *Hautarzt.*, 49, 6, 1998.
14. Kuncheva M. et al.: Emulsifying power of mannan and glucomannan produced by yeasts. *Int. J. Cosmet. Sci.,* 29, 377, 2007.
15. Lalor CB., Flyn GL., Weiner N.: Formulation factors affecting release of drug from topical formulation. 1. Effect of emulsion type upon in vitro delivery of ethyl p-aminobenzoate. *J. Pharm. Sci.,* 83, 1525, 1994.

16. Lin W., Lin S., Shu S.: Comparison of analysis of surfactants in cosmetics using High-Performance Liquid Chromatography and High-Performance Capillary Electrophoresis. *J. Surfact. and Detergents.*, 3, 67, 2000.
17. Manea A. E. et al.: Study of Cosmetic Creams Stability as a Function of Temperature. *Chem. Bull. "POLITEHNICA" Univ*., 53, 1, 2008.
18. Oyedeji F., Okeke I.: Comparative analysis of moisturizing creams from vegetable oils and paraffin oil. *J. App. Sci.*, 5, 157, 2010.
19. Ozcan M. M.: Some nutrional characteristics of fruit and oil of walnut (Juglans regia L.) growing in Turkey. *Iran J. Chem. Eng.*, 28, 57, 2009.
20. Taleb A., Eros I.: Rheological studies of creams. Part 2. Effect of water content on rheological characteristics. *Acta Pharm. Hung.*, 66, 71, 1996.
21. Taleb A., Eros I.: Rheological studies of creams. Part 2. Effect of lipophilic phase on consistency. *Acta Pharm. Hung.*, 66, 77, 1996.
22. Tamburic S.: Aging of polymer stabilized creams: rheological viewpoint. *C.&T.*, 115, 43, 2000.
23. Thiele J.J, Swarna E.M.: Vitamin E in human skin: Organ-specific physiology and considerations for its use in dermatology. *Molecular Aspects of Medicine*, 28, 646, 2007.
24. Tsamouris G., at al.: Lipid anaylis of greek walnut oil (Juglans regia L.). *Z. Naturforsch.*, 57, 51, 2002.
25. Park Ch., Chu W., Lee S.: Emulsion stability of cosmetic creams based on water-in-oil high internal phase emulsions. *Korea-Australia Rheol. J.*, 15, 125, 2003.
26. Patras A., Dorobantu P.: Physical and chemical composition of some walnut (Juglans regia L.) biotypes from Moldavia. *Lucrări Ştiintifice,* 53, 57, 2010.
27. Realdon N. et al.: Influence of processing conditions in the manufacture of O/W creams: I Effect on dispersion grade and rheological characteristics. *Farmaco*, 57, 341, 2002.
28. Realdon N., Ragazzi E. Morpurgo M.: Influence of processing conditions in the manufacture of O/W creams: II. Effect on drug availabity. *Farmaco*, 57, 349, 2002.