

EVALUATION OF ACRYLATES/C10-30 ALKYL ACRYLATE CROSS-POLYMER MIXTURE EFFECTIVENESS ON O/W TYPE EMULSION FORMULATION

EDITA KIZEVICIENE^{1*}, LAIMUTE JONAITIENE² and RIMANTAS PECIURA¹

¹Department of Drugs Technology and Social Pharmacy, Lithuanian University of Health Sciences, Medical Academy, Kaunas LT-50161, Lithuania

²Department of Pharmaceutical technician, Kaunas University of Applied Sciences, Kaunas LT-44162, Lithuania

Abstract: Acrylates/C10-30 alkyl acrylate cross-polymer mixtures were used in order to prepare stable and oil-in-water (o/w) pharmaceutical emulsions of organic oils. The resulting o/w pre-emulsions, prepared with different alkyl acrylate cross-polymers were unequally stable. We observed a synergistic effect when a polymer mixture was used and the resulting pre-emulsions were more stable for significant duration. The observed effect is confirmed by statistical analysis, the feature is remarkably important when we look for more stable pharmaceutical emulsions formed with less additives.

Keywords: cross-polymer mixture, synergistic effect

Oil-in-water emulsions are present in natural and mineral oils based products, including emollients and pharmaceuticals. For many of these sensitive substances emulsion formulating without heating is the only choice, therefore, emulsifiers such as acrylate C10–C30 alkyl–acrylate cross polymers – that can be used with any oil phase and can be easily prepared without heating of ingredients (1-4) were used. Very important indicator of the emulsion quality is stability, therefore, acrylate C10–C30 alkyl–acrylate cross polymers are suitable as they act both as primary emulsifiers and viscosity enhancing agents (3, 5, 6). Several investigations of the characteristics of biosurfactant mixtures (7) and the properties of various copolymeric emulsifier blends (8) confirm a synergistic effect when the emulsifier mixtures were used and the resulting emulsions were stable for several days. Our additional investigation of surface and interfacial tension, the determining parameters affecting the size distribution of the droplets (9) of copolymers of poly (acrylic) acid single emulsifiers and their mixtures, confirmed this effect. In connection with these results the interaction of cross-links between different copolymers of poly (acrylic) acid seems possi-

ble. Another explanation of these effects can be significant changes of surface and interfacial tension of polymer mixtures compared to single polymers (10-13). Therefore, we decided to investigate the mixture of copolymers of poly (acrylic) acid to form an o/w emulsions on the base of natural, vegetable oils, widely used in pharmaceutical industry and in manufacturing of individual medicines in pharmacies worldwide. To characterize prepared emulsion systems the method of determination of emulsion droplet size and droplet volume distribution was chosen.

The aim of this work was to investigate the effect of polymeric emulsifiers – acrylate C10–C30 alkyl–acrylate cross polymers (single ones and mixtures) on droplet size distribution of resulting o/w emulsions. The effect of the mixtures of these polymeric emulsifiers on droplet size distribution was investigated on emulsions prepared with an olive oil. Surface tension and interfacial tension between liquid phases of polymer mixtures were measured in the presence of several vegetable oils. To evaluate droplet size distribution of prepared pre-emulsions an image analysis method was used and the results were converted to volume basis (14).

* Corresponding author: e-mail: editakizevicius@gmail.com

EXPERIMENTAL

Materials

We used olive oil (Henry Lamoti oils (Bremen, Germany) as a dispersed phase, distilled water as the continuous phase, and high molecular weight copolymer of acrylic acid as well as long chain alkyl methacrylate crosslinked with allyl ethers of pentaerythriol – Pemulen™ TR1 NF and Pemulen™ TR2 NF (CTFA/INCI, according to IUPAC nomenclature system, general structure of Pemulen is considered as prop-2-enoic acid (3); Advanced Materials, Inc., (Calvert city, KY, USA) as the emulsifiers, sodium hydroxyd was supplied by E. Merck (Darmstadt, Germany). The base case emulsion consisted of 10, 25 and 50 wt % oil and from 0.02 to 0.4 wt % single polymeric emulsifier and their mixture (1 : 1), with the remainder water. Relatively high oil and low polymer concentrations were used to maximize the possibility of transdermal bioavailability of biological active substances dissolved in oil phase and to avoid the human adverse reactions to emulsifier. Interfacial tension between liquid phases of polymer mixtures were measured in the presence of castor oil (Roth), Karlsruhe, Germany; sunflower oil (Natura), AGD, Zarate (Argentina); olive oil (Henry Lamoti oils (Bremen, Germany) and liquid paraffin Brentag (Poland).

Emulsion preparation

All samples were prepared at ambient temperature ($20 \pm 2^\circ\text{C}$) by direct procedure: the polymeric emulsifiers were dispersed in the purified water phase containing neutralization base (sodium hydroxyd to final pH 6.0–6.5) by mixing. The calculated amount of the oil was slowly added and mixing was continued using stator-rotor device IKA® Eurostar 200 digital at 800 rpm for 15 min. Five passes were performed and approximately 0.0045 ± 0.0005 g of every pre-emulsion was sampled for measuring the droplet size distribution immediately after emulsion was formulated and 1 h, 2 h, 3 h and 24 h thereafter. To evaluate optimal quantity of

emulsifier, series of emulsions with different concentration of Pemulen™ TR1 NF, Pemulen™ TR2 NF (PTR1; PTR2) and their mixtures (PTR1 + PTR2) were prepared (Table 1). The effectiveness indicator was an absence of two separate layers of samples after 24 h (Tables 2-4).

Evaluation of droplet size distribution

Typically spherical shape droplet size was measured by image analysis and conversion from number to volume was made (14). Oil phase droplet size was measured with a 400 magnification coupled optical microscope (Motic BA310) and fixed using digital camera Motic Image Plus 2.0 M/L. Several photos were taken by the microscope from different parts of the samples (15). Automated determination of particle size was used. Triplicate measurements were made, and average specific surface area and variance were calculated (16), the coefficient of variation ($\text{std. dev./mean} \times 100$) was less than 10%.

Density measurement

Density of w/w polymeric dispersions and oils was measured using the glass ball probe supplied by the Sigma 702 instrument, with an accuracy of 10^{-4} g/cm³. Measurements were performed in an environment where temperature was ($22 \pm 2^\circ\text{C}$), and air pressure was 101.325 kPa.

Surface tension measurement

Surface tension of liquids was measured using the Attension Sigma 702 tensiometer (Biolin Scientific, Finland). The Sigma 702 was designed for measuring surface tension and interfacial tension between two liquids with Du Noüy ring and platinum plate. The method using Du Noüy involves slowly raising a platinum-iridium ring through the liquid until it detaches from the surface, taking into account Hugh Masons correction. For compatibility with earlier results Zuidema-Waters correction was used. After measuring, Du Noüy ring was immersed in ethanol and water, burned

Table 1 Composition of prepared o/w emulsions.

Emulsions series A	Emulsions series B	Emulsions series C
Water	Water	Water
Olive oil	Olive oil	Olive oil
Pemulen™ TR1	Pemulen™ TR2	Mixture (1 : 1) of Pemulen™ TR1 and Pemulen™ TR2
Sodium hydroxide	Sodium hydroxide	Sodium hydroxide

Table 2. Effective lowest concentrations of Pemulen® TR1 NF, Pemulen® TR2 NF and their mixtures to form stable Ol. Olivarum, 10%, (O/W) emulsion.

Ol. Olivarum, 10%, (O/W) Emulsion		Layer separation after 24 h, emulsifier PTR1	Layer separation after 24 h, emulsifier PTR2	Layer separation after 24 h, emulsifier PTR1 / PTR2 (mixture 1 : 1)
Total emulsifiers, wt%	Disperse phase, wt%			
0.4	10	-	-	-
0.2	10	-	-	-
0.1	10	-	-	-
0.05	10	-	-	-
0.03	10	+	-	-
0.02	10	+	+	-
0.01	10	+	+	+

Table 3. Effective lowest concentrations of Pemulen® TR1 NF, Pemulen® TR2 NF and their mixtures to form stable Ol. Olivarum, 25%, (O/W) emulsion.

Ol. Olivarum, 25%, (O/W) Emulsion		Layer separation after 24 h, emulsifier PTR1	Layer separation after 24 h, emulsifier PTR2	Layer separation after 24 h, emulsifier PTR1 / PTR2 (mixture 1 : 1)
Total emulsifiers, wt%	Disperse phase, wt%			
0.4	25	-	-	-
0.2	25	-	-	-
0.1	25	-	-	-
0.075	25	+	-	-
0.05	25	+	+	-
0.036	25	+	+	-
0.03	25	+	+	-/+

Table 4. Effective lowest concentrations of Pemulen® TR1 NF, Pemulen® TR2 NF and their mixtures to form stable Ol. Olivarum, 50%, (O/W) emulsion.

Ol. Olivarum, 50%, (O/W) Emulsion		Layer separation after 24 h, emulsifier PTR1	Layer separation after 24 h, emulsifier PTR2	Layer separation after 24 h, emulsifier PTR1 / PTR2 (mixture 1 : 1)
Total emulsifiers, wt%	Disperse phase, wt%			
0.4	50	-	-	-
0.2	50	-	-	-
0.15	50	-	-	-
0.1	50	-/+	-	-
0.05	50	+	-/+	-
0.04	50	+	+	-
0.036	50	+	+	-/+

with gas flame (1000°C) for a short time (5s). A platinum-iridium ring (Du Noüy) dimensions: R (ring) = 9.545 mm (wire) = 0.185 mm.

Measurements were performed in an environment where temperature was (22 ± 2°C), and air pressure was 101.325 kPa.

Interfacial tension measurement

Interfacial tension between two liquids was measured using Du Noüy ring. The platinum ring was preferentially moisturized by the heavier liquid. A ring from platinum was lowered and immersed into the lower layer phase (water, or polymeric PTR1, PTR2, PTR1/PTR2 dispersion). A liquid depth was regular, of 14 mm. Oily phase was carefully placed on the top of the water (polymeric PTR1, PTR2, PTR1/PTR2 dispersion) layer. The difference in the values of the density of measuring liquids were calculated using the following equation:

$\rho_{\text{heavy phase}} - \rho_{\text{light phase}}$. The ring was pulled up until the lamella from the interface broke off. At this stage the measured force gives interfacial tension value. All measurements were performed in an environ-

ment where temperature was $22 \pm 2^\circ\text{C}$ and air pressure was 101.325 kPa.

Statistical analysis

Statistical analysis of additional experiments was performed by two way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests using the software package Prism v.5.04 (Graph Pad Software Inc., La Jolla, CA) and Microsoft Excel software. A value $p < 0.05$ has been taken as the level of significance.

RESULTS AND DISCUSSION

We prepared sample emulsions at the basic conditions using emulsifiers PTR1, PTR2 separate-

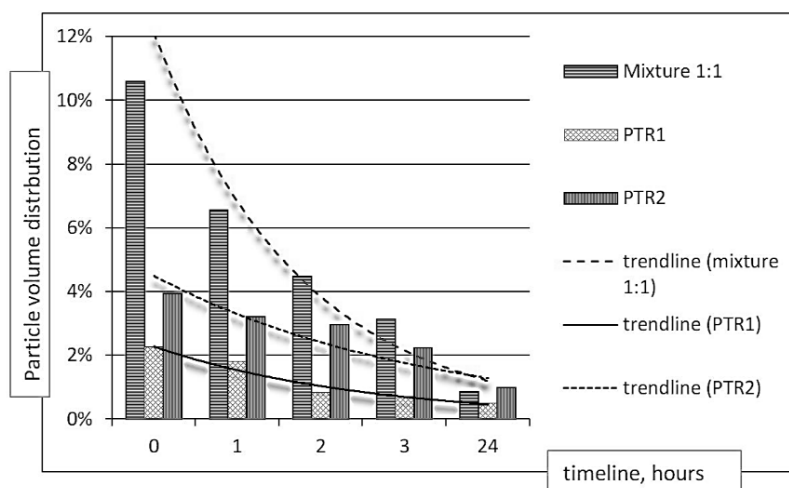


Figure 1. Volume growth dynamics of particles smaller than 10 μm of perimeter.

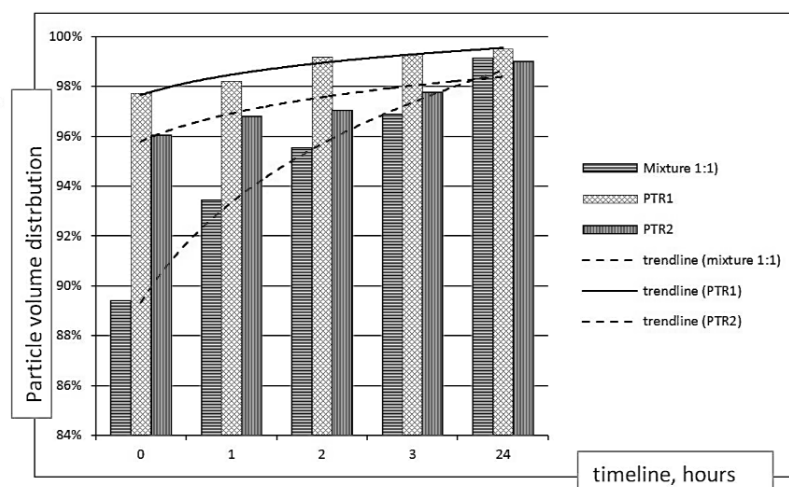


Figure 2. Volume growth dynamics of particles bigger than 10 μm of perimeter.

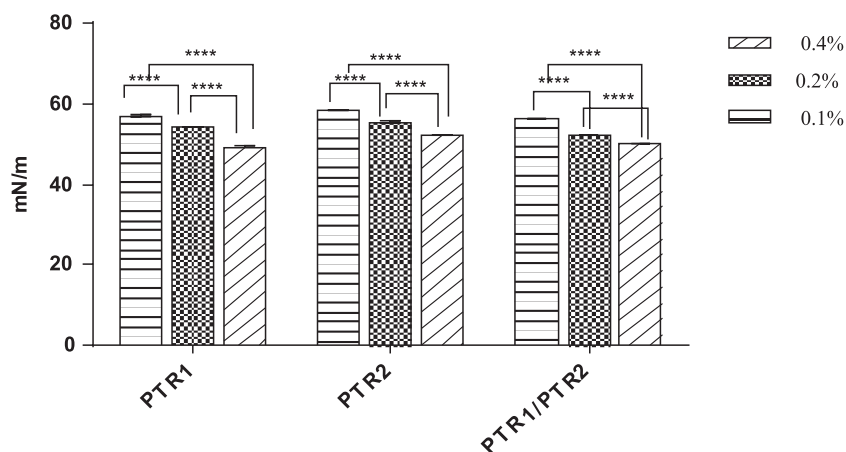


Figure 3. PTR1, PTR2 and (PTR1 + PTR2) w/w dispersions surface tension *versus* concentration, n = 5

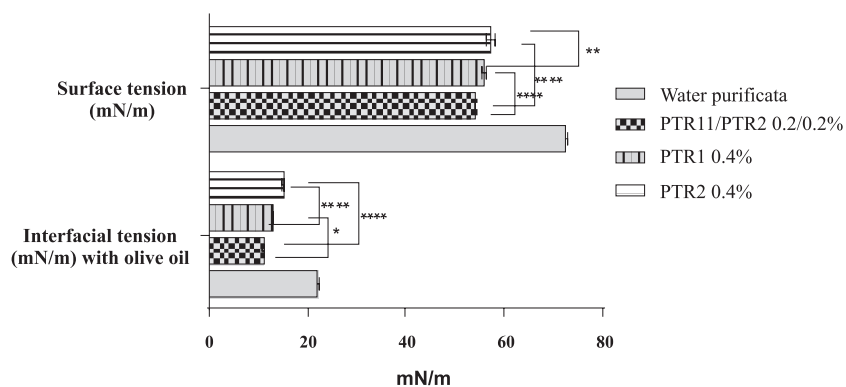
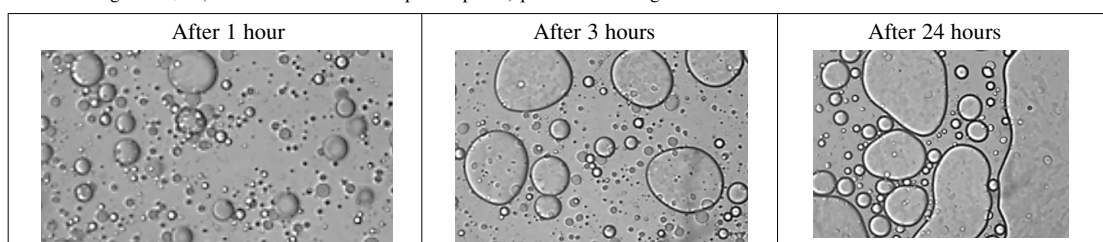


Figure 4. PTR1, PTR2 and (PTR1 + PTR2) w/w dispersions surface tension, interfacial tension of olive oil /aqua purificata and olive oil / PTR1 + PTR2 w/w) dispersions, n = 5

Table 5. Images of o/w (OliOlivarum 50 wt% dispersed phase) pre-emulsion degradation.



ly and a mixture of both emulsifiers (1 : 1). Every emulsion was prepared three times repeatedly, and the volume of emulsion droplets distribution was measured to ensure data reproducibility. The reproducibility of experimental procedure and the distribution measurements were confirmed by the maximum standard deviation (about 10%) in the measured droplet size distribution based on five repetitions for any sample of pre-emulsions. Dynamics of

o/w pre-emulsion, prepared on olive oil basis, degradation was investigated as an oil phase droplets coalescence. Images of oil droplet size growth in emulsion with disperse phase 50 wt % is shown in Table 5.

The droplet size was measured immediately after preparation of pre-emulsions and, thereafter, 1, 2, 3 and 24 h, subsequently; obtained data was converted to volume basis. The dynamics of oil

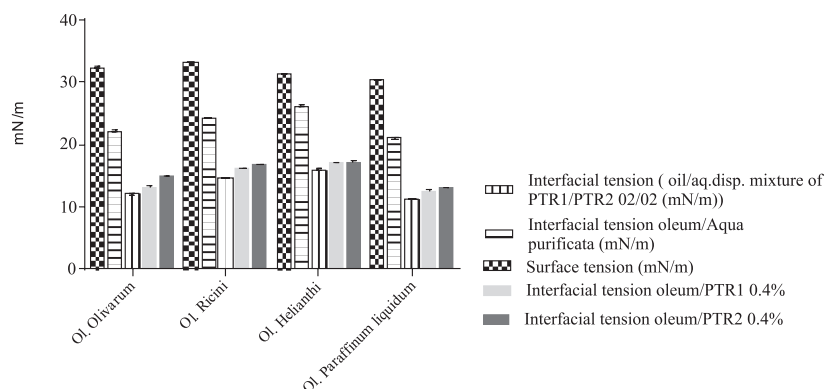


Figure 5. PTR1, PTR2 and (PTR1 + PTR2) w/w dispersions surface tension, interfacial tension of oil /aqua purificata and oil /PTR1, PTR2 or PTR1 + PTR2 w/w) dispersions, n = 5

droplets coalescence is shown in Figures 1, 2. Figure 1 shows a change of the total volume of oil particles with perimeter $10.0\ \mu\text{m}$, Figure 2 shows a change of total volume of oil droplets with perimeter $10.0\ \mu\text{m}$ and more. Whereas the effective concentration of PTR1 + PTR2 mixture in emulsion system is significantly lower (0.04 wt%, total, instead of 0.1 wt% for PTR2 and 0.15 % for PTR1), emulsifier mixture demonstrates a clear synergistic effectiveness to form a bigger volume of small oil droplets.

Previous studies confirmed that due to their surfactant nature *Pemulens*, like other polymeric emulsifiers, migrated toward the interface and showed low surface activity (17, 18). Surface tension of PTR1, PTR2, PTR1/PTR2 aqueous dispersions and interfacial tension of PTR1, PTR2, PTR1/PTR2 aqueous dispersions / olive oil was observed and evaluated (Figures 3, 4). When the quantity of the polymers PTR1, PTR2 is increased (0.1 – 0.4 wt%), surface tension decreases significantly ($p < 0.05$) and surface tension w/w dispersions of mixtures PTR1 + PTR2 is significantly lower in w/w dispersions of PTR1, PTR2 separately ($p < 0.05$) which has been clearly shown by the surface tension results (Fig. 3). PTR1 and PTR2 aqueous dispersion of surface tension differ significantly (PTR1 lower than PTR2, $p < 0.05$), however, previous studies have shown that the surface tension with PTR1 and PTR2 are similar (18). Interfacial tension between the mixture of an aqueous dispersion of emulsifiers PTR1 + PTR2 / olive oil was significantly ($p < 0.05$) lower compared to an aqueous dispersion of PTR1 or PTR2 separately / olive oil (Fig. 4). This phenomena was confirmed by the interfacial measurements using different oils (Fig. 5). The interfacial tension reduction some-

what justify the effectiveness of emulsifier PTR1/PTR2 mixture to form more stable emulsion systems compared to when PTR1 and PTR2 are used separately. It was observed that the mixture of polymeric emulsifiers PTR1/PTR2 reduces particle size by suppressing droplet coalescence and by aiding droplet breakup through reduced interfacial tension.

CONCLUSION

Synergistic effect of polymeric emulsifiers PTR1 and PTR2 mixtures was observed. The effective concentration of acrylate C10–C30 alkyl–acrylate cross polymer mixtures in emulsion system can be significantly lower compared to emulsions, prepared with a single polymeric emulsifier. This effect is remarkably important when looking for stable pharmaceutical emulsions formed with less additives.

Acknowledgment

The authors wish to express their acknowledgement to the Advanced Materials, Inc., (Calvert city, KY USA) for benevolent provision of Pemulen samples.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Yassin G.E.: Br. J. Pharm. Res. 4, 1014 (2014).
2. Traynor M.P., Burke R., Frias J.M., Gaston E., Barry-Ryan C.: Int. Food Res. J. 20, 173 (2013).

3. Tegeli V., Thorat Y.S., Shivsharan U.S., Gajeli G.T., Kumbhar S.T., Chougule G.K.: *Med. Chem. Res.* 2, (2011).
4. Szucs M., Sandri G., Bonferoni M.C., Caramella C.M., Vaghi P., Szabó-Révész P. et al.: *Eur. J. Pharm. Sci.* 34, 226 (2008).
5. Shahin M., Hady S.A., Hammad M., Mortada N.: *Drug. Dev. Ind. Pharm.* 37, 559 (2011).
6. Hemker W.: *SÖFW Seifen, Öle, Fette, Wachse* 116, 505 (1990).
7. Cerón-Camacho R., Martínez-Palou R., Chávez-Gómez B, Cuéllar F., Bernal-Huicochea B. et al.: *Fuel (Lond)* 110, 310 (2013).
8. Martin J.D., Velankar S.S.: *J. Rheol. (N Y)* 51, 669 (2007).
9. Kryukova E., Sister V., Rustambekov M., Ivannikova E.: *J. Pet. Sci. Eng.* 50, 766 (2015).
10. Sing C.E., Zwanikken J.W., de la Cruz M.O.: *J. Chem. Phys.* 142, 1 (2015).
11. López-Barrón C.R., Macosko C.W.: *J. Rheol. (N Y)* 58, 1935 (2014).
12. Moncho-Jordá A., Rotenberg B., Louis A.A.: *J. Chem. Phys.* 119, 1266 (2003).
13. Lyu S. J.T., Bates F.S., Macosko C.W.: *Macromolecules* 35, 7845 (2002).
14. Burgess J., Duffy E., Etzler F., Hickey A.: Particle size analysis: AAPS Workshop report, cosponsored by the Food and Drug Administration and the United States Pharmacopeia. *AAPS J.* 6, (3), 23 (2004).
15. Varka E.M.: *Open J. Appl. Sci.* 2, 139 (2012).
16. Dapčević H.T., Dokić P., Krstonošić V., Hadnašev M.: *Eur. J. Lipid Sci. Technol.* 115, 313 (2013).
17. Bobin M.F., Michel V., Martini M.C.: *Colloids Surf. A Physicochem. Eng. Asp.* 152, 53 (1999).
18. Simovic S., Tamburic S., Milic-Askračić J., Rajic D.: *Int. J. Pharm.* 184, 207 (1999).

Received: 29. 04. 2016