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The Isolation of Ricinoleic Acid from Castor Oil by Salt-solubility-based Fractionation for the Biopharmaceutical Applications

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Abstract The goal of this work was to develop a method for the preparative isolation of ricinoleic acid (RA) from Castor oil (CO) for use as building blocks in biopharmaceutical synthesis. The separation efficiency achieved with the impurity extraction approach (IEA) and the salting-out approach (SOA) based on utilization of monobasic alkali salt of RA was examined. SOA showed better separation efficiency. Fractional precipitation from the co-solvent system consisting of isopropyl ether:ethanol (IPE:EtOH) 65:35 v/v and applied at a CO: co-solvent system mass: volume ratio of 1:5 produced a high quality purified RA (purity 97.9–98.6%). It contains residuals of linoleic acid and oleic acid as the main impurities and satisfies all requirements of the official compendia for oleaginous vehicles for parenteral administration. The total yield of this process was 55.5 ± 2.5% and the recovery of RA was about 70% (calculated relatively to the yield of the crude product). The study of the synthesis kinetics of RA oligomers and of the biopolymer prepared from these oligomers indicated that mono-functional fatty acids (FAs) affect the composition of the oligomers' mixture and delay chain elongation of both RA oligomers and biopolymer. The developed novel process is simple, flexible, highly reproducible, inexpensive, and has high scalability potential.

Keywords Fatty acids purification · Fractional precipitation · Salt-solubility · Castor oil · Ricinoleic acid · Quality assessment · Natural sources for biopharmaceutical synthesis

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Introduction

Ricinoleic acid (RA) is a C18 FA with a double bond at the C(9) position and a hydroxyl group at the C(12) position (*cis*-12-hydroxyoctadeca-9-enoic acid) [1, 2]. It is obtained from castor oil (CO), a mixture of triglycerides predominantly containing a RA-acyl moiety (85–90%) [1, 2]. The other fatty acids (FAs), which account for about 10–15% of triglycerides in CO, are generally oleic acid (18:1(9)), linoleic acid (18:2(9,12)), linolenic acid (18:3(9,12,15)), palmitic acid (16:0), stearic acid (18:0), eicosanoic acid (20:0) and dihydroxystearic acid. CO is one of the few commercially available glycerides that contain hydroxyl functionality in such a high percentage of a single FA.

CO and its main compound have an extensive use in many fields of chemical synthesis utilized in industrial processes [1]. RA has been also used for the synthesis of biodegradable polymers developed specifically to be used as implantable drug delivery matrices for the treatment of various pathological conditions [3, 4]. Mono-functional FAs act as chain terminators to yield low-molecular-weight polymers with high polydispersity [3, 4]. Thus, successful isolation of RA from mono-functional FAs may result in high molecular weight polymers. Different techniques have been proposed for the separation of a single FA from a FA mixture including: solvent extraction, distillation, fractionation via urea-inclusion compounds (UIC), counter-current distribution (CCD), counter-current chromatography (CCC), HPLC and others [5].

RA decomposes upon distillation or polymerize unless the hydroxyl group and/or carboxyl group are protected [2, 6]. Distillation of RA esters obtained by protecting the carboxylic group with aliphatic alcohols was found to be ineffective for RA fractionation from linoleic and oleic acids [7]. Moreover, the attempts to increase the inter-molecular

mass differences between the RA and non-hydroxylated FAs (NHFAs) by acetylating the hydroxyl moiety of aliphatic esters of RA with acetic anhydride resulted with insufficient purity (less then 95%) and low yield. Crystallization of the free FA (FFA) form and methyl ester of RA requires large amounts of organic solvents and utilization of specially designed low temperature regimes [6]. Furthermore, it is characterized by lower separation efficiency between RA and other unsaturated FAs. Fractionation via UIC also does not lead to the effective isolation of RA from other components of the FFA fraction derived from its natural source [8, 9].

Liquid chromatography using a solid stationary phase is an effective method in terms of purity and yield. However, it is not practical for large scale purification processes due to high cost [10]. Successful isolation of the RA methyl ester by CCD was achieved by Berdeaux et al. [11]. This process is characterized by good yield and high purity of the isolated compound. Nevertheless, in non-automated laboratory practice that requires routine, very frequent, isolation of RA this approach is markedly laborious because of the multiple extraction operations and the necessity to collect and treat numerous fractions.

Fractionation by crystallization based on the salt-solubility approach was also applied for preparative isolation of RA from CO [12–14]. This approach has advantages in laboratory and industrial practice for preparative separation due to its potential operation simplicity and because it may not require substantial capital cost investment if designed properly [15]. However, this separation technique was employed in the past usually as a route for isolation of RA required for investigations that are not related to separation process or as pre-purification step. Consequently, it was not part of these studies and was suited mainly for low-scale laboratory applications; the reports do not include assessment of reproducibility, and they give varying estimations of the separation efficacy [12–14]. In general, the assessment of the quality of purified product from a safety point of view is lacking.

The aim of this study was to develop a scalable method for isolation of high quality RA by the salt-solubility approach for potential application in the biopharmaceutical synthesis. The design and selection of a purification scheme was based on considerations of efficiency of isolation, scalability, reproducibility, cost of materials and operating cost, as well as simplicity and flexibility [9, 10]. The estimation of the quality of purified RA was performed in accordance with safety considerations. These evaluations include the study of the technological processes' impact on the capability of the purified product to meet the requirements for the oleaginous vehicle for parenteral administration.

Experimental Procedures

Materials

CO European Pharmacopoeia (Eur Ph) was obtained from Florish (Haifa, Israel), CO USP from Sigma-Aldrich Ltd. (Rehovot, Israel) and CO Purum from Fluka (Rehovot, Israel). EtOH, methanol (MeOH, HPLC grade), ethyl acetate (EtAc), IPE, hydrochloric acid (HCl), acetonitrile (HPLC grade), *n*-hexane, *n*-heptane, chloroform and acetic anhydride were purchased from BioLab (Jerusalem, Israel). Potassium hydroxide (15% water content) (KOH), anhydrous magnesium sulfate ($MgSO_4$), and phosphoric acid (87%) were obtained from Frutarom (Haifa, Israel). Palmitic acid (16:0) (99%), vaccenic acid (18:1(7)) (97%), 9,10-dihydroxystearic acid (98%), linolenic acid (18:3(9,12,15)) (99%), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilane, sebacic acid (99%), *p*-toluene sulfonic acid (97%), Celite® 521, 1-naphthylamine, oxalylchloride, dideuterium oxide (D_2O), hexadeuterodimethyl sulfoxide (DMSO-d_6), phenolphthalein and activated charcoal (Nurit®) were obtained from Sigma-Aldrich Ltd. (Rehovot, Israel). RA (99%), oleic acid (18:1(9)) (99%), stearic acid (18:0) (99%) and linoleic acid (18:2(9,12)) (99%) were purchased from ICN Biomedicals Inc. (OH, USA).

All solvents were of analytical grade if not specified otherwise above and were used without further purification.

Preparation of FFA Fraction

CO (250 g) was hydrolyzed by refluxing with KOH alcoholic solution (60 g dissolved in 500 ml of EtOH) for 1 h in an oil bath. EtOH was evaporated and the residue dissolved in 1.2 l of deionized water, and acidified with concentrated HCl to pH = 1. The liberated FAs were extracted with 600 ml of EtAc. The organic layer was dried over $MgSO_4$, decolorized with Nurit®, filtered and EtAc evaporated using an evaporator on a water bath (50 °C). The oily residue was clarified according to approach of Gulbekian and Skellon [12] with slight modifications. It was mixed with *n*-hexane at a ratio of 1:5 w/v and placed at −4 °C for 72 h at darkness. The resultant biphasic system with solid precipitate concentrated mainly at the interface was vacuum filtered at ambient temperature through a 0.22 μm PTFE membrane with a 47-mm diameter (I.S.I. Ltd., Petah-Tikva, Israel) protected with a 0.5 g Celite® 521 (550 g/m^2). The organic solvent was evaporated under vacuum in a rotary evaporator at 60 °C over a water bath. The FFA fraction was then analyzed by GC-MS and HPLC.

Analysis of Solid Matter Separated from FFA Fraction

The solid matter was washed with *n*-hexane, collected from the filter surface and dissolved in MeOH. The solution was filtrated and evaporated to dryness. Then it was converted to ethyl ester using *p*-toluene sulfonic acid as a catalyst. The unmodified solid matter and its ethyl ester were converted to trimethylsilyl (TMS)-derivates and analyzed by GC-MS. The unmodified solid matter was also examined by direct injection of the solution in MeOH (100 ng/ml) to Finnigan LCQ DUO mass spectrometer (TermoQuest, San Jose, CA, USA), running on a negative ion mode. ^{1}H-NMR spectra were obtained on a Varian 300 MHz spectrometer in 5 mm OD tubes. DMSO-d_6 containing tetramethylsilane served as solvent and shift reference. The D-H exchange experiment for attribution of the signals of the hydroxyl groups' hydrogens was performed by addition to the solution of the analyzed compound in DMSO-d_6 of one drop of D_2O and following signals disappearance. In the second set of examinations the solid matter was washed with *n*-hexane, collected from the filter surface and then extracted three times with 50 ml of chloroform. The collected extracts were combined, concentrated by evaporation to a volume of 1 ml and examined by GPC.

RA Purification: Impurity Extraction (IEA) Versus Salting-out (SOA)

CO was hydrolyzed as described above. In the impurity extraction approach (IEA), the EtOH solution of hydrolyzed CO was evaporated until the EtOH/solids content (v/w) was 20%. The precipitate was then dispersed at a ratio of 1:5 w/v (CO: co-solvent mixture) in a number of co-solvent systems consisting of IPE and increasing amounts of EtOH (taking into account the amount of EtOH already present in the precipitate). The dispersions were equilibrated at ambient temperature for 1 h with constant shaking (100 rpm). In the salting-out approach (SOA) predetermined amounts of EtOH were evaporated from the ethanol solution of hydrolyzed CO. IPE was introduced to concentrates heated to approximately 50–55 °C and the obtained dispersion was vigorously shaken. It was left for 24 h at ambient temperature in the dark covered by a thermo-protective jacket. The solid phase was separated from the liquid phase by centrifugation at 4.0×10^3 rpm for 5 min, followed by 7.5×10^3 rpm for 90 min ($r = 6.5$ cm), with the rotor operating temperature at 16 °C. The centrifugation was performed in six 250 ml high-density polyethylene centrifugation bottles equipped with polypropylene screw caps (Nalge Nunc International, NY, USA). The supernatant liquid phase was decanted. The compressed precipitate of potassium salts of FAs was mechanically separated from the color bodies' enriched fraction that segregated in the distinct phase (lower phase). The FFAs were liberated from the compressed raffinate by acidification with 1 N HCl and then extracted with EtAC. The resultant organic extracts were treated according to the procedure described above adhering in addition to following condition. The evaporation of organic solvents (EtAC and *n*-hexane) was performed in a 2 l round bottom flask to ensure a thin-layer shape of the purifying product when exposed to a gentle temperature (60 °C) under vacuum [16]. Samples obtained during these trials were examined by GC-MS and HPLC.

Determination of Sedimentation Volume

Dry precipitate was obtained after digestion of CO with KOH and evaporation of EtOH to dryness. Precipitate containing residual EtOH was prepared by the evaporation of EtOH until a precipitate containing 20% v/w of EtOH was obtained. Five grams of dry precipitate and 5.8 g of precipitate containing residual EtOH were transferred to 30 ml cylindrical glass bottles and dispersed manually in a number of co-solvent systems at a ratio of 1:5 w/v (CO: co-solvent system) after hermetic sealing of the bottle with a septum. The dispersion of precipitate obtained by partial evaporation of EtOH was performed taking into account the amount of EtOH already present in the precipitate. All dispersions were equilibrated at ambient temperature for 1 h with constant shaking (100 rpm) and, then allowed to settle in the dark. The volume of the sediment was recorded after 24 h of settling at ambient temperature. The average ($n = 6$) sedimentation volume, $F(\%)$, was calculated as a ratio of the equilibrium volume of the sediment (V_u) to the total volume of the suspension (V_0). The kinetics of change of sedimentation volume with time was studied similarly recording the volume of the sediment immediately, after 2, 24 and each 24 h for additional 6 days. The sedimentation volume of the dispersions prepared according to the SOA was examined on preparative scale.

Morphological Characterization of Dispersed Phase

Aliquots (5 ml) of dispersions of crude potassium ricinoleate in co-solvent systems were smeared in a very thin layer on the filter paper and the liquid phase was removed by vacuum filtration. Separated solid matter was dried for 72 h under reduced pressure over phosphorous pentoxide.

The dry samples were gold-coated and then imaged using Qanta 200 SEM (FEI, Eindhoven, The Netherlands).

Solid Phase Residual Solvent

Dispersed solid matter was separated from solvents by centrifugation as described above. The weights of the wet precipitate and the dry precipitate after solvent evaporation were recorded ($n = 6$). The residual solvent content in the precipitates was calculated as the difference between the weight of wet and dry precipitate. It was corrected for residual solvent in the dry precipitate as determined by thermogravimetric analysis (TGA). The calculated residual solvent content was confirmed by TGA. TGA was performed utilizing a Melter TG50 module calibrated with standard weights. Samples weighing 30.00 ± 3.00 mg were placed in alumina crucibles equipped with pinholed covers and scanned at a heating rate of 10 °C/min under nitrogen flow of 1 ml/min.

Chromatographic Analysis

GC-MS measurements were performed in a Trace MS detector (TermoQuest Finnigan) operating in a positive electron impact (EI^+) mode of ionization, connected to a Trace GC 2000 Series (TermoQuest CE Instruments, Milan, Italy) equipped with a ZB-5 Phenomenex® (Torrance, CA, USA) capillary column (30 m × 0.25 mm; 0.25 μm film thickness) with helium as the carrier gas at constant flow 1 ml/min. The full scan acquisition was performed in the range of 50–600 amu with 1.7 scans/s and the separation was performed employing temperature gradient elution mode [8]. Samples were converted to TMS-derivates with BSTFA + 1% trimethylsilane for 40 min at 70 °C. HPLC-UV was carried out using an HPLC system consisting of an HP 1050 quaternary pump, an HP 1050 auto-sampler with 200 μl loop, and an HP 1050 Photodiode Array Detector (PDA) coupled with HP ChemStation for LC 3D Systems indented for data processing (Agilent Technologies, Palo Alto, USA). The separation was performed on C18 RP analytical columns (LichroCart® 250-, Lichrospher® 100, 5 μm), which was protected with a C18 RP guard column (LichroCart® 4-4, Lichrospher® 100, 5 μm). Unsaturated FAs, including the compound of interest RA, were determined employing a mixture of 70% acetonitrile/30% water/0.2% phosphoric acid at a flow rate of 1.0 ml/min as eluent, with UV detection at 205 nm [17]. The quantitative determination of the saturated FAs such as palmitic acid, stearic acid and 9,10-dihydroxystearic acid was carried out according to the method of Sparreboom and coworkers using 1-naphthylamine pre-column derivatization [18].

Analysis of Purified RA

Chromatographic methods were performed as explained above. The refractive index was determined using a calibrated Abbe Refractometer (Bausch&Lomb, NY, USA). The optical rotation was measured with a polarimeter (Optical Activity Ltd., England) at the sodium D-line, $c = 5$ g/100 ml in acetone. The iodine value, peroxide value and unsaponifiable matter were determined according to USP [19a]. The acid value was assessed by titration of samples dissolved in ethanol with an ethanol solution of potassium hydroxide using phenolphthalein as the indicator [20]. Water content was determined by the Karl–Fisher method with the Karl–Fisher reagent (Hydronal—Composite 5, Riedel-de Haen) potentiometrically using a double Pt wire electrode connected to a 796 Titroprocessor (Methohm, Herisau, Switzerland) for data recording and processing. The clarity, color and odor assessment was performed organoleptically [21]. Monitoring of the polymeric compounds' presence in the purified product was performed by GPC using the equipment and conditions described below. The following parameters were determined by Aminolab, an external analytical laboratory (Ness Ziona, Israel): heavy metals content was determined using ICP, residual solvents by GC-MS—Head Space and residual on ignition by thermo-gravimetry.

Polymer Synthesis and Molecular Weight Determination

Oligomers of RA and poly(sebacic acid-*co*-ricinoleic acid) copolymer (p(SA-RA) 3:7) was prepared as previously described by melt condensation [3, 4]. At different time points samples were withdrawn from reaction pool and molecular weights of the oligomers and polymers were estimated by gel-permeation chromatography (GPC) [3, 4].

Results and Discussion

The optimized process of RA isolation from CO by salt-solubility-based fractionation is depicted in Fig. 1. To obtain high purity RA two fractionation steps are required, which are performed after oil hydrolysis. The first separation step is a salt-solubility-based fractionation of RA from NHFAs and the second step is intended for the fractionation of 9,10-dihydroxystearic acid in the free acid form from the purified RA. The developed technological process also includes two auxiliary steps for the purified product's decolorization based on segregation and adsorption phenomena, respectively. The purification algorithm described in Fig. 1 was designed using the results of the laboratory work as a guideline for its development. The following

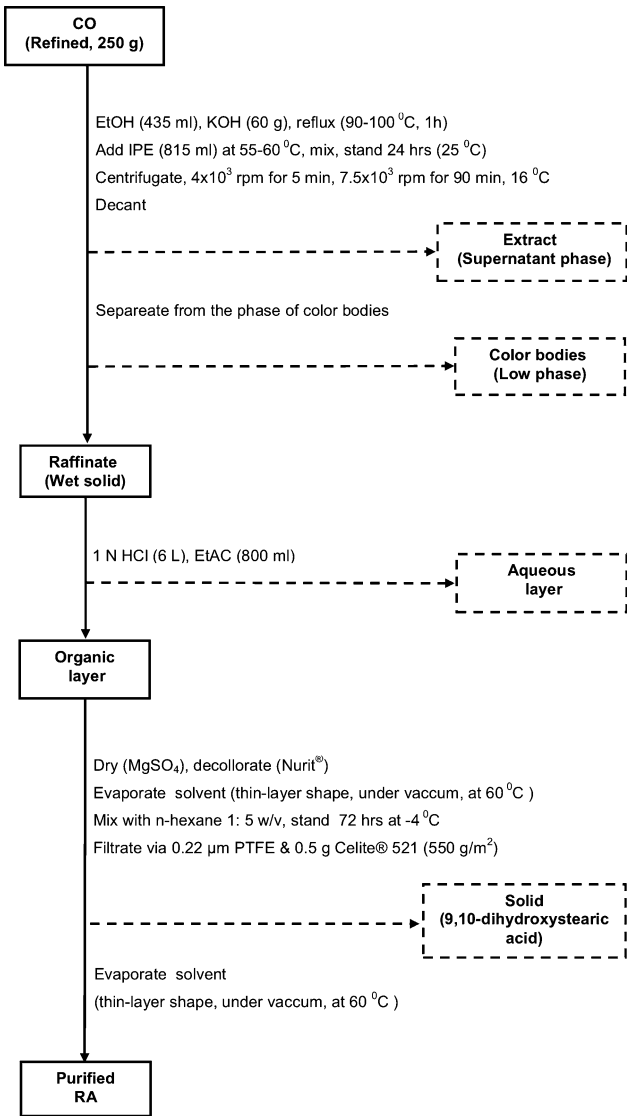

Fig. 1 Flow scheme for preparation of purified RA from CO by fractional precipitation utilized a salting-out approach. *CO* castor oil, *EtAC* ethyl acetate, *EtOH* ethanol, *HCl* hydrochloric acid, *IPE* isopropyl ether, *KOH* potassium hydroxide, *RA* ricinoleic acid

sections of this report present the obtained results, outline the guidelines used for the algorithm development, and overview the developed technology.

FA Composition of the FFA Fraction Derived from CO

GC-MS analysis was performed on the TMS-derivates of the decolorized and clarified FFA fractions derived form CO to determine their composition. RP HPLC was employed for FA quantification. These examinations revealed that the qualitative profiles of NHFAs of all the examined COs include palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1(9)) and its isomer—vaccenic acid (18:1(7)), linoleic acid (18:2(9,12)) and also linolenic acid (18:3(9,12,15) (Table 1). Quantitative determination of oleic acid and its positional isomer—vaccenic acid—was performed cumulatively, since these isomers were not resolved using an RP HPLC analytical method.

The solid matter responsible for the clouding of the FFA fraction, which was derived from the examined COs, was fractionated by selective precipitation from *n*-hexane. This fractionation yielded 0.65–0.75% w/w of a white crystalline solid with a paraffin odor. GC-MS analysis of its TMS-derivate, showed that is eluted in single peak and the fragmentation pattern indicated that its structure is consistent with that of 9,10-dihydroxystearic acid. It was in accord with the high cleavage probability of the chemical bounds which are adjacent to the OTMS groups giving the characteristic fragmentation ions *m/z* 215 and *m/z* 317 resulted from the cleavage toward the methyl end of the chain and the carboxylic end, respectively [22]. GC-MS analysis of TMS-derivate of the ethyl ester of this compound revealed a similar fragmentation pattern. The mass-difference between the observed *m/z* ratios for molecular ions $[M]^+$, the fragments (*m/z* 390 vs. *m/z* 346) attributed to TMS-rearrangement of the esters of oxygenated FAs, and the fragments (*m/z* 317 vs. *m/z* 273) that resulted from the cleavage toward carboxylic end [22], proved the presence of the single carboxyl group in the molecular structure.

^{1}H NMR (DMSO-d_6) was also consistent with the elucidated structure: 0.84 ppm (t, 3H, terminal –CH_3), 1.23–1.45 ppm (two overlapping ms, 26H, aliphatic hydrogens), 2.17 ppm (t, 2H, –$\underline{CH_2}$–CO–OH), 3.20–3.50 ppm (br, 2H, –$\underline{CH}$(OH)–$\underline{CH}$(OH)–), 3.90–4.60 ppm (br, 2H, –CH($\underline{OH}$)–CH($\underline{OH}$)–), 11.81 ppm (br, 1H, carboxylic acid hydrogen). The respective mass obtained after direct injection into the mass spectrophotometer running on a negative ion mode corresponded to the deprotonated molecule $[M\text{-}H]^-$, ion at *m/z* 315, which matches that of the 9,10-dihydroxystearic acid. To our knowledge this is first published report that assigns the exact position of hydroxyl moieties in dihydroxystearic acid occurring in CO.

The qualitative and quantitative composition of the FFA fractions derived from the examined COs were comparable (Table 1) and were also in good agreement with the published data [2]. Therefore, further development was based on utilization of Eur Ph CO if not specified otherwise in the text.

Dispersion Characteristics of Crude Potassium Ricinoleate

Examination of the morphology of the dispersed phase revealed that in all cases it consisted of irregular or

Table 1 FAs compositions of decolorized and clarified FFAs fraction derived from refined COs

Parameter	CO Eur. Ph (Florish, Israel)	CO USP (Sigma, Israel)	CO Purum (Fluka, Israel)
Yield (wt%)	87.0	86.1	86.5
FFA composition	Weight percentage		
16:0	1.10	1.00	1.10
18:0	1.05	1.00	1.10
18:1(9) + 18:1(7)	4.90	4.87	4.60
18:2(9,12)	5.20	4.95	4.85
18:3(9,12,15)	0.30	0.25	0.31
RA	87.50	87.90	88.10

Decolorization was performed by treating the ethyl acetate solution of FFAs fractions derived from COs with a suspension of activated charcoal. FFAs fractions derived from COs were clarified by fractionation at −4 °C of the 9,10-dihydroxystearic acid in its FFA form from a mixture of neutralized CO alkali digest and *n*-hexane at ratio 1:5 w/v. The component identities were specified by GC-MS and quantitative determination was performed by RP HPLC

CO castor oil, *FAs* fatty acids, *FFAs* free fatty acids, *RA* ricinoleic acid

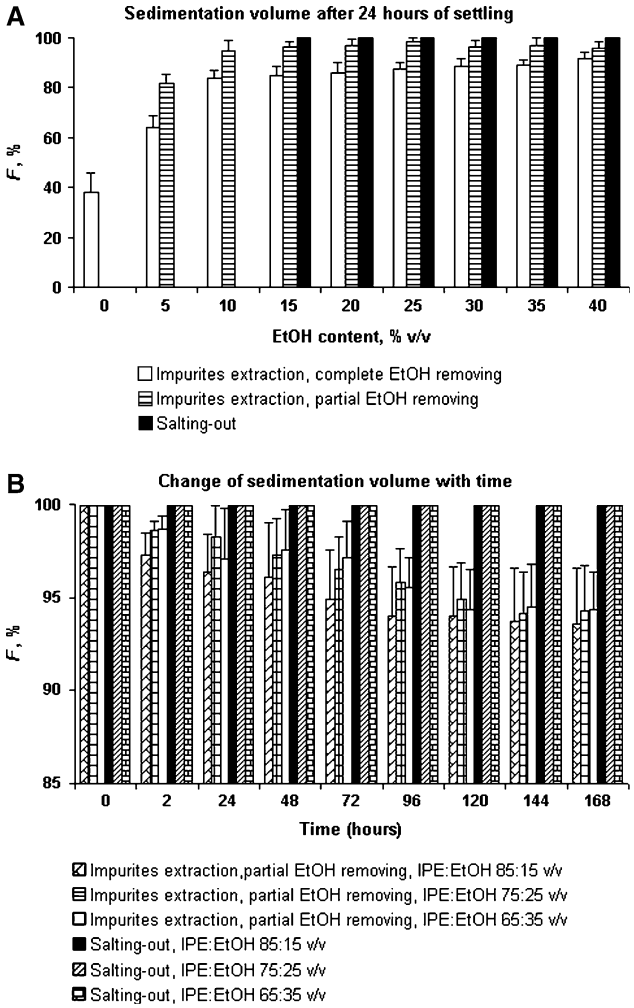

Fig. 2 **a** Impact of the EtOH content and disposition on the sedimentation volume (*F*, %); **b** changes with the time of the sedimentation volume (*F*, %) of the dispersion of CO alkali digest in the co-solvent mixtures. *Bars* represent SD ($n = 6$)

flake-shape particles with a low order of organization and non-uniform relief. The volume occupied by the dispersed phase after 24 h of settling, *F*(%), was found to be dependent on the EtOH content in the dispersing media (Fig. 2a). Incorporation of EtOH into the co-solvent systems led to substantial increase of the observed sedimentation volume in comparison to the dispersion of completely dry CO alkali digest in IPE only. In the IEA over the EtOH concentration range of 5–35% v/v, the significantly higher values of *F*(%) were observed for dispersions prepared applying partial removing of EtOH in comparison to that prepared by dispersing the completely dry solid material ($p < 0.05$, two-tailed unpaired *t* test with Welch correction). All examined dispersions prepared according to the SOA had characteristic values of sedimentation volume ($F = 100\%$) regardless of the composition of dispersing phase. The study of the changes in sedimentation volumes with time revealed that dispersed systems prepared according to the IEA with partial EtOH removing and the SOA are extremely stable dispersions (Fig. 2b).

The solvent uptake by the dispersed phase had a similar pattern of the dependence on EtOH content in the applied co-solvent systems. EtOH incorporation into the dispersing media and increase of its concentration caused the residual solvent content of the solid phase to increase. It reached a maximum at 10% v/v of EtOH and above this concentration of EtOH remained stable, and was as high as 40% w/w (Fig. 3). This feature was independent of the applied co-solvent system composition and the dispersing approach ($p > 0.05$, Kruskal–Wallis test with Dunn's multiple comparisons test).

Yield and Separation Efficiency

Bivalent metal salts were applied in the past for isolation of RA by the salts-solubility approach [12–14]. These salts and particularly the most commonly used barium salt of RA give cake that is less compressible than alkali salts of RA. Even though it allows vacuum filtration for solid–liquid separation, the use of the bivalent metals creates mixed salts. This problem together with the inherent limitations of fractional precipitation discussed below often result in a remarkable decrease in separation efficiency. As a consequence, several re-crystallizations from EtOH followed by extraction with boiling diethyl ether (DEE) were needed to improve separation [13].

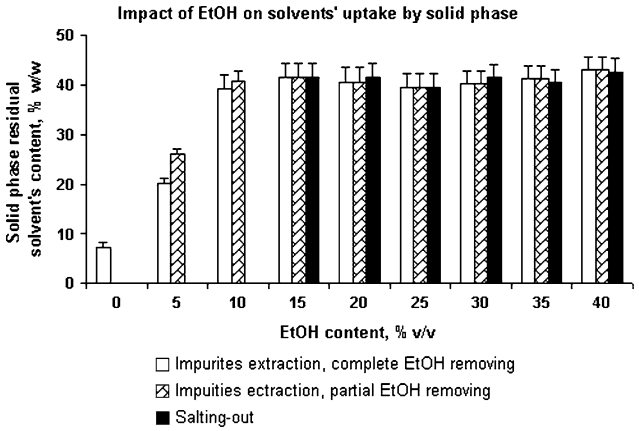

Fig. 3 Solid phase residual solvent as function of EtOH content in the co-solvent mixture. *Bars* represent SD ($n = 6$)

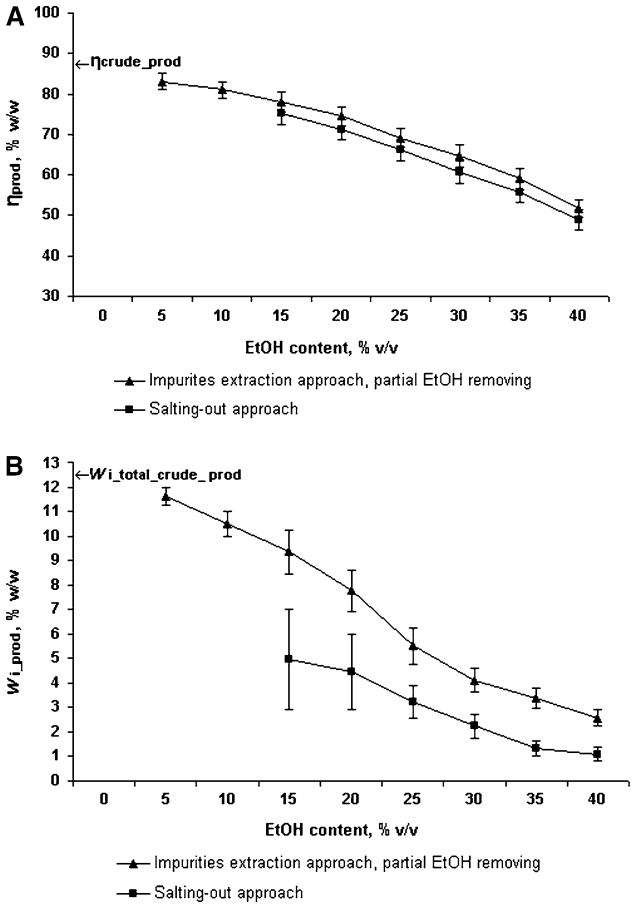

Fig. 4 **a** Yield (η_{prod}, %), and **b** NHFAs content in the purified product (w_{i_prod}, %) as a function of EtOH content in a binary co-solvent system consisting of IPE and EtOH. η_{crude_prod} (% w/w) and $w_{i\_total\_crude\_prod}$ (% w/w) notate the yield of FFA fraction derived from CO and the total content of NHFAs in this fraction, respectively. *Bars* represent SD ($n = 6$)

To overcome the problem of the mixed salt formation, the monovalent metal was applied as counter ion. The separation of RA from NHFAs in the developed method is based on the same principle of differential saturation of the solution by solutes as in the case of bivalent counter ions. The design of the fractional precipitation includes controlling the composition of the co-solvent system and the mass-to-volume ratio between the mass of raw material and the volume of the co-solvent system at desired temperatures. Manipulation of these parameters allows adjustment of the process to concrete operation conditions, thus reflecting its flexibility.

Incorporation of EtOH into the co-solvent system had an impact on both yield and separation efficiency (Fig. 4). The yield and the NHFAs content in the purified product were inversely proportional to the EtOH content in the co-solvent system (Fig. 4a). Over the same concentration range of EtOH that was applied in the SOA (15–40% v/v), the differences in the applied operation conditions between two examined approaches had no impact on the yield ($p > 0.05$, two-tail Mann–Whitney test). On the other hand, the significantly higher separation efficiency for the same yield was achieved using the SOA (Fig. 4b) ($p < 0.05$, two-tail Mann–Whitney test). In the SOA increasing the EtOH content from 35 to 40% v/v in the co-solvent system was not found to be cost-effective. It did result in the statistically insignificant improvement of impurity removal ($p > 0.05$, two-tail Mann–Whitney test), did not reach the lower variability in the impurity content, and led to a decrease in yield.

The NHFAs profiles of the purified product obtained by IEA with partial EtOH removal and by the SOA are presented in the Table 2. Experiments with the SOA produced a wider range of the raw data representing each FA contribution in comparison to IEA. But, with an increase in the EtOH content in the co-solvent system this variability decreased. The comparison of profiles showed that the average relative contribution of each specified FA to the total content of NHFAs in the obtained products had remained stable and independent of the operation conditions. As shown in Table 2, these average contributions exhibited nearly identical values to the relative abundance of respective FAs in the NHFAs content of the crude product. This finding demonstrates that none of the potassium salts of NHFAs showed a tendency for preferential implantation into or exclusion from the raffinate phase. On average, the amount of each compound examined decreased proportionally to the total reduction of NHFAs content that occurred with the increase of EtOH concentration in the co-solvent system (Fig. 4, Table 2). On the basis of these results, the implantation probability of the potassium salt of NHFAs into the solid phases was found to be independent of their structure (presence or absence of double bonds in the lipidic moieties, their number and the hydrocarbon chain length).

Table 2 Abundance of particular NHFAs in the total NHFAs content of purified RA (w/w, %) by IEA with partial EtOH removal and SOA

NHFA	Starting CO[b]	Approach													
		IEA[a]								SOA					
		Co-solvent system composition: IPE:EtOH v/v								Co-solvent system composition: IPE:EtOH v/v					
		95:5	90:10	85:15	80:20	75:25	70:30	65:35	60:40	85:15	80:20	75:25	70:30	65:35	60:40
16:0	8.76	9.21 (0.76)	8.90 (0.79)	9.48 (0.69)	7.87 (0.63)	9.01 (0.66)	9.22 (0.72)	8.67 (0.58)	8.84 (0.61)	8.52 (3.53)	7.64 (3.00)	8.90 (1.86)	8.76 (1.90)	9.12 (1.88)	8.58 (2.13)
18:0	8.37	7.80 (0.61)	7.15 (0.66)	7.71 (0.56)	8.26 (0.63)	7.99 (0.59)	7.93 (0.55)	8.20 (0.64)	7.62 (0.58)	7.90 (3.28)	7.10 (2.76)	8.57 (1.67)	8.14 (1.71)	8.00 (1.75)	7.77 (1.93)
18:1(9) + 18:1(7)	39.04	37.00 (1.25)	39.01 (1.87)	37.93 (3.62)	42.19 (4.16)	37.96 (4.87)	41.39 (4.50)	38.90 (4.66)	39.45 (5.07)	38.04 (15.68)	39.26 (13.52)	39.25 (9.22)	40.68 (9.01)	38.47 (8.57)	40.25 (9.70)
18:2(9,12)	41.43	43.60 (1.33)	42.63 (2.02)	42.59 (3.97)	39.64 (4.31)	42.48 (5.51)	39.29 (4.13)	41.61 (4.98)	42.00 (5.43)	43.16 (18.95)	43.63 (16.27)	41.13 (8.60)	40.10 (9.22)	42.06 (9.32)	41.08 (10.18)
18:3(9, 12,15)	2.40	2.39 (0.07)	2.31 (0.11)	2.29 (0.22)	2.04 (0.22)	2.56 (0.33)	2.17 (0.25)	2.62 (0.27)	2.09 (0.27)	2.38 (0.99)	2.37 (0.82)	2.15 (0.45)	2.32 (0.50)	2.35 (0.52)	2.32 (0.57)

Bars represent SD ($n = 6$)

CO castor oil, *EtOH* ethanol, *IEA* impurity extraction approach, *IPE* isopropyl ether, *NHFA* non-hydroxylated fatty acid, *RA* ricinoleic acid, *SOA* salting-out approach

[a] In IEA the partial EtOH removing approach was applied for preparation of the precipitate of CO alkali digest to facilitate its dispersion in extracting media. The conditions are specified in the "Experimental Procedures" description

[b] Starting CO is a castor oil of European Pharmacopoeia grade obtained from Florish (Haifa, Israel), and the reported values of particular NHFA abundance in the total NHFAs content in the starting material was calculated on the basis of data presented in Table 1

In the IEA, the delay in the leaching of impurities from the solid matter results in the lower efficiency of NHFAs removal in comparison to the SOA. Impurities' inclusion in the solid phase in the IEA is predetermined by operation conditions due to transformation of the entire alkali digest of CO to a solid state, while the mechanisms of their implantation into the solid phase in SOA are not so obvious and require clarification. When a substance crystallizes from a multi-component solution, it is usually contaminated to some extent by other substances whose concentrations in the precipitate and the initial solution can have a wide range of values [23]. As seen from the data presented in Table 2 the concentration of each particular impurity in the solid phase varies from lot-to-lot for the same operating conditions, indicating that 24 h is insufficient for establishing true equilibrium in the system. In this case, the incorporation of impurities into the solid phase should be considered as the result of co-precipitation, rather than phase distribution taking place under equilibrium conditions [23].

Co-precipitation is a complex process that goes through a combination of a number of mechanisms. They include formation of an independent solid phase by impurities when their concentration is sufficiently high in the initial solution [23]. However, such a process is highly unfavorable in the described system because impurities, such as potassium salts of NHFAs, are to a large extent isomorphous with the main substance especially in respect to the poorly structured lattice of its deposit. Thus, formation of an independent solid phase by these macro-impurities might be overlooked. An isomorphism phenomenon is the basis of one of the most rational interpretations of the findings present in Fig. 4 and Table 2. These results strongly suggest that the implantation of the potassium salts of NHFAs into the solid phase in SOA is ruled by the precipitation rate of a macro-component of which one of the main determinates is a co-solvent system composition. Hence, co-crystallization—a process of the capture of impurities by the growing lattice of the macro-component's deposition or adsorption on its surface—might be considered as a mechanism of co-precipitation in the present case of SOA [15, 23].

On the basis of the results obtained, the most effective SOA, i.e. IPE:EtOH 65:35 v/v (Fig. 4), was selected for isolation of RA. The following steps of the purification algorithm were modified. The volume of EtOH used for CO hydrolysis (500 ml) was decreased to 435 ml and the EtOH evaporation step was omitted. The CO hydrolysis was conducted for 1 h after the system became homogeneous. The reaction vessel was removed from the heating bath and IPE (835 ml) introduced to the constantly stirred alkali digest of CO at 55–60 °C (Fig. 1).

Characterization of Purified Product Quality

Medicinal grade CO is regarded as a well-tolerated and non-toxic, biocompatible compound and it is approved as a pharmaceutical excipient for parenteral, eternal and topical routes of administration [24]. Thus, it is essential to show that the developed technological process does not lead to worsening product quality and consequently will not increase the likelihood of any possible health implications associated with parenteral administration of articles based on RA purified by this method. Vegetables oils have a long history of pharmaceutical applications. Consequently, for this class of oleaginous vehicles for parenteral administration the guidelines for identification of the particular hazards related to technological process and the reference values for risk acceptance criteria are well established by the authorized compendia constraints.

The mass-balance analysis revealed that three groups of major impurities may be identified in the composition of purified RA obtained by optimized SOA. They include low amounts of naturally occurring FAs, residuals of water and residuals of organic solvents (Table 3). The degree of overall purity taking into account the content of residuals of water and residuals of organic solvents was 97.9–98.5%. The FFA fraction consisted of 98.3–98.9% of RA and the remainder consisted of linoleic acid (18:2(9,12)), oleic acid (18:1(9)) and oleic acid isomer (18:1(7)) as the main impurities, with traces of palmitic acid (16:0), stearic acid (18:0), linolenic acid (18:3(9,12,15)) and 9,10-dihydroxystearic acid.

The residuals of organic solvents may have serious health implications. Therefore this issue required particular consideration in order to estimate the necessity for introducing an auxiliary technological step or optimization of operation conditions to decrease their levels. Two of the applied solvents, EtOH and EtAC, are related to Class 3 solvents which are considered of low risk to human health. The FDA-recommended concentration limit for these solvents is 5,000 ppm, and the purified product fulfills this requirement. IPE is related to Class 4 organic solvents, for which no adequate toxicological data on which to base a permitted daily exposure were found. Its level in the final product was below the detection limits (<5 ppm). *n*-Hexane belongs to Class 2 organic solvents, whose content should be limited in pharmaceutical products. The recommended concentration limit for *n*-hexane is 290 ppm. The residual of this solvent in the purified product was 2.6–2.8 times less. Moreover, after 4 h of polymer synthesis under vacuum, the levels of EtAC are decreased in the synthesized polymers below detection limits (<10 ppm), and *n*-hexane content decreased to 5 ppm. This indicates that the current process is sufficient for ensuring low content of

Table 3 The quality specifications of the purified RA isolated from refined COs by the optimized approach (depicted in Fig. 1)

Profile of main impurities		Low content potential leachables		Non-selective bulk analytical characteristics		
Parameter	Value	Parameter	Value	Parameter	Value	Reference value[b]
FA profile, % w/w		Peroxide value, mequiv/1,000 g	0.997 ± 0.003	Acid value (FFA content)	187.55 ± 0.22	187.96
16:0	0.09 ± 0.02	Heavy metals, % w/w		Iodine value	85.54 ± 0.15	85.05
18:0	0.10 ± 0.01	Pb	<0.00002[a]	Refractive index (20 °C)	1.4715	1.4716
18:1(9) + 18:1(7)	0.55 ± 0.07	Hg	<0.00002[a]	Optical rotation (22 °C)	+6.70°	+6.67°
18:2(9,12)	0.65 ± 0.08	Cu	<0.00002[a]	s.p./m.p.	4 °C/5 °C	4 °C/5 °C (standard)
18:2(9,12, 15)	<0.01	As	<0.00005[a]			
9,10-dihydroxystearic acid	<0.01	Ash, w/w %	<0.01			
RA	98.24 ± 0.28	Unsaponifiable matter	Not founded			
Total FA content (% w/w)	≥99					
Water content (% w/w)	0.26 ± 0.04					
Residual solvents (RSs) (ppm)						
IPE	<5[a]					
EtOH	<50[a]					
EtAC	1,537 ± 78					
n-Hexane	107 ± 8					
Total RSs content (% w/w)	<0.17					

The data presents results obtained from characterization of the purified product isolated from raw materials that were purchased from three different vendors (specified in Table 1). Data is given with SD, $n = 12$ ($n = 4$, for every one of the three raw materials used). The yield was 55.5 ± 2.5% in all cases

Abbreviations: the same as in Fig. 1, Tables 1 and 2

[a] The value was below the Detection Limit specified in the table for each respective parameter

[b] The Merck Index, An encyclopedia of Chemicals, Drugs, and Biologicals, 1996, p. 1413

organic solvent residuals in both purified monomer and synthesized polymer.

The additional quality assessment comprised estimation of products of the oils' autoxidative deterioration including polymerization by-products [6, 12, 25] and determination of the potentially toxic leachables such as heavy metals and inorganic contaminants.

The first step in the characterization of unsaturated FAs autoxidative deterioration was determination of the peroxide value of the purified monomer. Peroxide value of the raw materials were in the range of 3–4 mequiv/1,000 g [3.236 ± 0.006 (SD, $n = 6$, intra-batch, 3.436 ± 0.007 (SD, $n = 6$, intra-batch, 3.943 ± 0.005 (SD, $n = 6$, intra-batch,), for CO Eur. Ph (Florish Israel), CO USP (Sigma, USA) and CO Purum (Fluka, USA), respectively] and decreased to level of 1 mequiv/1,000 g in the isolated RA (Table 3). This level is ten times smaller than the limit specified by USP for safflower oil and soybean oil, permitted for parenteral administration including i.v. injection and the limit specified by Eur Ph for CO [19b, c, 24].

The spectral properties of the constituents of purified RA separated by RP HPLC were studied in order to detect and monitor the compounds that are structurally related to secondary products of the oils' autoxidative deterioration. All components of purified RA had typical UV absorption spectra for non-conjugated unsaturated FAs (λmax ~ 200 nm). The peak purity assessment ruled out a co-elution of unidentified compounds having characteristic adsorption spectra that distinguish them from non-conjugated unsaturated FAs [17, 20].

The decrease of peroxide value supposes peroxide decomposition—a process involving free radical formation whose chain propagation step is responsible for acceleration of autoxidative deterioration of unsaturated FAs [25]. The results of the examination of the spectral properties of the constituents of purified RA suggests that unsaturated

FAs were not substantially involved in this process [17, 20]. This finding is supported by the absence of peroxide decomposition products such as dicarboxylic acids and non-naturally occurring oxygenated FAs in the purified RA and the crude FFAs faction derived from CO [12–14]. Thus, it is reasonable to conclude that the initial peroxide location, as well as the suggested decomposition and oxidative structure modification related to autoxidative process were restricted mainly to the color bodies' fraction. Natural pigments protect oils from oxidative damage acting as free radical scavengers, chain-breaking antioxidants, and/or by quenching the oxidative reactions [26]. CO and many of its derivatives are stabilized by the hydroxyl group, which is beta to the double bond. This hydroxyl group protects the double bond by preventing the formation of peroxides [1]. The autoxidation of RA requires the use of catalyst even under the condition of active oxygen supply and heating [12]. However, autoxidative deterioration of non-hydroxylated acids such as monoenoic, dienoic and especially trienoic FAs is a cause for concern because of their higher susceptibility to autoxidation and cross-linking [16].

Three types of examinations were applied for detection of polymerization by-products. The first one was monitoring of polymeric compounds by GPC in the purified monomer. The second analytical attitude was the same, but instead of examining the purified monomer directly the concentrated chloroform extract of solid mater obtained during insolubilization in *n*-hexane was investigated. These examinations did not indicate the presence of polymerization by-products in the purified monomer (data not shown). The third approach was examination of non-selective bulk analytical characteristics of the purified product that have been found to be sensitive indicators of the presence of polymerized compounds and also overall quality of the purified RA [6, 7, 12–14]. These characteristics included total FA content, iodine value, refractive index, optical rotation, as well as solidification point and melting point. This evaluation did not reveal any unexpected deviations from the reference value that were inconsistent with the stated quality established by mass-balance analysis (Table 3). The contents of heavy metals and inorganic contaminates were below detection limits (Table 3). These detection limits were far from the levels that are considered to have adverse health implications [19b, c].

The purified monomer at ambient temperature appeared to be a water-like, perfectly clear, and free of foreign bodies, oily liquid. It has a slight, but characteristic odor indicating the presence of residual EtAC, and has no odor suggesting rancidity [21]. It is also completely colorless when inspected in low amounts, whereas larger bulk amounts had a slight yellowish shade. In addition, no evidence of unsaponifiable matter was found in the purified monomer when the extraction approach was applied with subsequent gravimetric determination (Table 3).

Influence of Mono-functional FAs on Polymer Synthesis

The influence of mono-functional FA presence (that are NHFAs) on the synthesis of biodegradable polymer materials was examined by following the formation of RA oligomers and the synthesis of the poly(ester-anhydride) of RA and sebacic acid at a ratio of 70:30—p(SA:RA) 30:70 (Fig. 5). The formation of RA oligomers was determined by GPC. The rate of monomer disappearance in the case of purified RA was higher than the consumption of FAs present in the crude product (Table 4). The amount of remaining monomers in the case of purified RA after 28 h of reaction was 2.5 times smaller than in the case of the crude product. In order to achieve the same level of monomer consumption, an additional 20 h were needed. In addition, the synthesis from the purified RA resulted in higher content of undecamers and lower octamer content in comparison to that obtained for crude RA. The observed differences can be explained by one-sided blocking of chain elongation by mono-functional FAs, and therefore decreasing the probability of chain-elongation.

The copolymer p(SA:RA) 30:70 was synthesized from oligomers prepared from both crude and purified RA. The molecular weight change was followed for 120 h. As can be seen, the Mw was affected by the purity of RA while the Mn was almost similar (Fig. 6). Insertion of RA monomer and poly(RA) oligomers into the poly(sebacic acid) involved two steps: (a) a random introduction of ester bonds along the polyanhydride chain by reaction of the last with a hydroxyl moiety of RA, and (b) polymerization of these oligomers to high molecular weight [4]. In the second stage, chain elongation delay was prominent until 70 h of polymerization. Polymer prepared from oligomers of crude RA needed additional 50 h to gap the difference in the Mw.

The calculated mole ratio between the free hydroxyls and anhydride bonds revealed that there is excess of the latter as a result of hydroxyl group participation in the

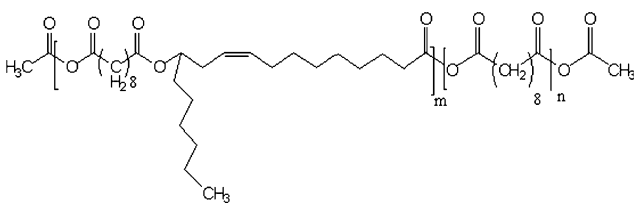

Fig. 5 Structure of poly(sebacic-ricinoleic ester-anhydride) (*p*(*SA-RA*))

Table 4 Distribution of oligomers in reaction pot during synthesis of poly(RA) oligomers (w/w, %) prepared from crude RA and purified RA

Oligomer length (number of FA moieties per chain)[a]	Monomer type															
	Crude RA									Purified RA						
	Time (h)									Time (h)						
	0	1	2	4	8	20	28	47	50	0	1	2	4	8	20	28
1	100	84.96 (5.25)	76.24 (3.81)	64.40 (3.54)	49.18 (2.16)	23.83 (1.66)	18.11 (1.01)	10.00 (0.60)	9.12 (0.64)	100	79.92 (3.99)	66.21 (3.31)	48.76 (2.44)	32.35 (1.62)	10.42 (0.52)	7.49 (0.37)
3		13.74 (0.79)	20.55 (1.03)	27.75 (1.39)	33.96 (1.77)	32.08 (1.43)	28.05 (1.50)	17.82 (0.86)	16.57 (0.98)		17.72 (0.89)	26.19 (1.31)	34.16 (1.71)	34.66 (1.73)	20.42 (1.02)	16.29 (1.38)
5		1.30 (0.08)	3.20 (0.17)								2.36 (0.15)	7.61 (0.32)				
6				7.84 (0.42)	16.87 (0.79)	23.69 (1.38)	23.83 (1.29)	19.66 (0.99)	19.29(0.88)				17.08 (0.85)	20.25 (1.02)	21.37 (1.07)	18.73 (0.94)
8						20.41 (1.11)	30.00 (1.32)	16.81 (0.96)	17.06(0.85)					12.49 (0.62)	47.79 (2.89)	7.48 (0.64)
11								35.57 (1.85)	37.96 (1.97)							50.01 (2.00)

Samples were withdrawn periodically from the reaction pot and analyzed by GPC. The NHFAs profile of the crude RA is given in Table 1 and the specifications of the purified RA are listed in Table 3. *Bars* represent SD (n = 3)

Abbreviations: the same as in Fig. 1, Tables 1 and 2

[a] The difference in the Mw of oligomers built from the same number of FA moieties, but of different structure, was considered as a case insensitive for GPC analysis, therefore the oligomer length was calculated assuming a neat poly(RA) structure for all synthesized oligoesters (terminated and non-terminated by NHFAs)

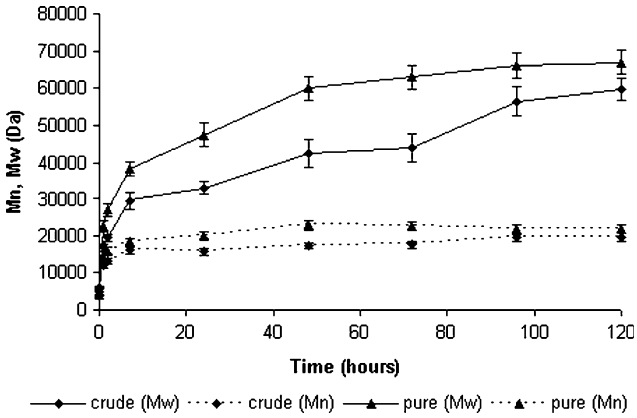

Fig. 6 Molecular weight change during the formation of p(SA-RA) using the oligomer mixture prepared from crude and purified RA. Samples were withdrawn periodically from the reaction pot and analyzed by GPC. The compositions of the oligomer mixtures that were utilized for synthesis are presented in the last time points in Table 4. *Bars* represent SD (n = 3)

formation of poly RA oligomers. Hence, during transesterification, free oligomers of bi-functional polyanhydride segments are also formed. These segments bridge between the carboxylic acid-terminated poly(anhydride-ester), leading to formation of the polymer with a similar Mw in both cases (Fig. 6).

An Overview of the Developed Technology

The developed purification algorithm is designed as a batch operation process (Fig. 1). It employs materials equipment and technological processes widely applied in chemical and pharmaceutical synthesis in industry and laboratory practice. It is simple to operate, flexible, and the capacity of equipment required for such process appears to be unlimited. This purification process may be easily automated and the capital cost for such technological attitude seems to be modest [9, 10].

It should be noted that use of the refined oils as the raw material might result in an increase in the cost of materials [9]. Nevertheless, the main guideline was a safety, therefore standardized medicinal grade CO was selected as the raw material due to its generally recognized safe (GRAS) status [24]. This choice was also important in order to prevent problems which lead to non-reproducible and confusing results occurring in the crystallization practice as a result of turning out the purest available raw material [9, 15]. CO is an inexpensive and environmentally friendly, naturally occurring renewable resource [1]. It is refined anyway to make it more resistant to deterioration during storage and for detoxification [2]. Thus, the selection of refined CO as raw material would not be expected to increase substantially the material cost.

Application of vacuum filtration in the examined case for solid–liquid separation revealed that the cake of dispersed potassium ricinoleate underwent fast compression on a preparative scale. It complicates the solid–liquid separation by filtration and requires the use of sophisticated approaches. On the other hand, this feature of the dispersed phase was employed for solid–liquid separation. Inducing cake sedimentation and compaction with centrifugal force provides good repeatability and assurance of process reliability. The main factors that determine compressibility of dispersed phase—particles' softness, deformability, adhesiveness and irregularity of the shape—are independent of the variations in the applied operation conditions. It is determined by the nature of the isolated compound such as the *cis*-conformation of the lipidic moiety of RA, its higher relative contribution to the molecular weight of potassium ricinoleate in comparison to the mass-contribution of the alkali cation, and, obviously, by incorporation of solvent molecules into the lattice of solid matter (Fig. 3).

The color bodies insoluble in the applied co-solvent system create a separate dispersed phase of viscous drops of semi-solid consistency. This dispersed semi-solid phase was separated from the suspended potassium salts of FAs by differential centrifugation. It was performed by controlling the pelleting efficiency of the rotor that results in segregation of the dispersed drops of insoluble color bodies into the distinct phase. The segregation process was based on the higher sedimentation velocity of the dispersed semi-solid drops in comparison to that of the suspended deposit of potassium salts of FAs. The consequent coalescence of the precipitated drops future contributed to the creation of a new segregated phase sediments below the raffinate of FA salts (Fig. 1). This attitude fits in well with the developed algorithm and together with the removal of color bodies soluble in the mixture of IPE:EtOH (present in withdrawing supernatant extract) improves the separation capability of the approach.

The 9,10-dihydroxystearic acid was fractionated by selective precipitation of the free acid form from *n*-hexane in the final steps of the purification process. Due to higher polarity of 9,10-dihydroxystearic acid in comparison to RA its complete removal was not achieved during the fractionation of potassium ricinoleate from NHFAs. Consequently, clouding of the FFA liberated from the raffinate took place. In the separation technology based on the salt-solubility concept separation of RA from more polar compounds than RA itself, for instance dicarboxylic acids of shorter hydrocarbon chain length or dihydroxystearic acid, usually requires an additional step of fractionation [7, 12–14]. Berdeaux et al. [11] reported that clouding of the methyl ricinoleate purified by CCD method was also observed and as a result additional step of extraction with *n*-hexane was necessary for clarifying the product.

The dependence of technological processes on many parameters might make it difficult to reproduce a process which is known to work and could make the transfer of a process to a new material much more difficult than it appears superficially [15]. Variability in system behavior can takes place mainly because of inconsistencies in the raw material and technological processes [9]. The variability introduced by macro-component content and macro-impurities composition of different examined raw materials was relatively low (Table 1). Due to co-precipitation phenomenon it will be always expected some content of impurities in the purified product (Fig. 4, Tables 2, 3). However, the FAs profile of the CO obtained from inbred cultivated *Ricinus communis* consists of the completely safe dietary FAs in addition to the main compound, thus this issue has no safety significance [1, 2]. The specification of the micro-impurity composition of raw materials, such color bodies, volatiles, unsaponifiable matter, heavy metals and other impurities of inorganic nature was not in the scope of present work. They were treated in bulk to attain maximum removal or prevention of introducing them through the process. This attitude could be considered as justified, since the system behavior and the low degree of variability in the results obtained with the examined group of raw materials does not indicate that this issue might have an impact on the purification outcomes (Table 3).

The main efforts during the development of the described purification algorithm were focused on decreasing the variability caused by inconsistency in the technological process. This was accomplished successfully (Table 3) by the selection of the co-solvent system with a high polar modifier concentration (35% v/v of EtOH) that ensures the lowest variations in the separation efficiency (Fig. 4b, Table 3). Moreover, the design of the separation technology was based on physicochemical processes that occur spontaneously and only limited intervention by the operator was required. Its function was restricted to solely unsophisticated and commonly applied manipulations (Fig. 1). Thus, there is little bias diversity introduced by different operators [15].

During development of a purification technology it is best to be guided by the attitude that it is "necessary to know exactly what is required" [15]. In cases where special pharmaceutical grades are needed, such as those needed for vegetable oils used to manufacture products intended for parenteral administration, special steps for quality assurance and quality measures are necessary [21]. On the other hand, overstated quality requirements that demand application of costly technological process may result in a high excipient price and, consequently, to the undesirable cost-ineffectiveness of the new pharmaceuticals. This study as well as the report of Berdeaux et al. [11] indicates that preparative production of a high quality monomer, such as

Table 5 The main differences among the developed process employing monovalent counter ion (MVCI) and previous processes using bivalent counter ion (BVCI) in the salt-solubility-based fractionation of RA from CO

Approach	MVCI		BVCI[a]	
Aspect[b]	Description	Comments	Description	Comments
Technological				
Counter ion	K^+	Source: KOH	Ba^{2+}	Source: $Ba(OH)_2$ and $BaCl_2$
Auxiliary step of conversion to the salt of the desired counter ion	Not required	The salt is already created during CO alkali digestion with KOH	Required	Performed after CO hydrolysis at different stages of the process by reaction of crude RA with $Ba(OH)_2$ in EtOH, alkali salt of crude RA with $Ba(OH)_2$ in EtOH, and by reaction of alkali salt of crude RA with $BaCl_2$ in water
Mass-transfer medium	Binary co-solvent system: EtOH:IPE	Applied once	Mono-component mass-transfer media: EtOH, DDW and DEE	Applied as a pure solvents at each distinct step of fractionation process
NHFAs separation	One-stage fractional precipitation	Fractional solidification of RA potassium salt from applied co-solvent system	Multi-stage fractional precipitation	Includes: (1) low temperature fractionation of saturated FAs from FFA fraction and then from its ethanolic solution, followed by (2) barium salts crystallization from aqueous solution of potassium ricinoleate, (3) its extraction with boiling DEE, and (4) several stages of re-crystallization from EtOH
Separation efficiency limiting processes	Co-precipitation and extract entrapment		Mixed salt formation, co-precipitation and extract entrapment	
Solid–liquid separation	Centrifuging and decanting	Utilizes the compressibility properties of potassium ricinoleate cake; employed also for segregation of raffinate from the insoluble fraction of color bodies	Vacuum filtration	Becomes feasible due to relatively higher stability of the barium salt of RA to compression induced by filtrate drag
Decolorization	Applied	Based on adsorption phenomenon; activated charcoal is used as a sorption agent	Not applied	Achieved during multiple re-crystallization
HFAs separation	One-stage fractional precipitation	Low temperature fractionation of 9,10-dihydroxystearic acid from biphasic mixture of RA and *n*-hexane	Separate fractionation stage is not included	Mainly occurs during low temperature fractionation of saturated FAs from FFAs fraction and then from its ethanolic solution; the other fractionation steps (following after low temperature fractionation) also contribute to HFAs separation, but with less efficiency
Quality assessment stage	Not required through the process run		Required through the process run	Employed for determination of the process end-point: used in decision making about a number of re-crystallizations from EtOH that shell be performed
Overall design	Two-stage fractional precipitation with decolorization	Simple, flexible, reproducible; relatively time- and labor-non-consuming process	Multi-stage fractional precipitation without decolorization	Relatively complicated: includes a number of intermediate steps, many fractionation stages, and quality assessment stage through the process; time- and labor-consuming process

Table 5 continued

Approach	MVCI		BVCI[a]	
Aspect[b]	Description	Comments	Description	Comments
Economical				
Material cost	Comparably lower		Comparably much higher	Main reason: required larger amount of solvents; minor reason: $Ba(OH)_2$ or $BaCl_2$ is more expensive than KOH and introduce the extra cost to that of KOH which is already utilized in the process
Operation cost	Comparably lower		Comparably much higher	Not only because it is a time- and labor-consuming process characterized by higher complexity, but also due to increase in the equipment maintenance cost and energy expenditure for one batch production
Safety				
Ether explosion hazard	Comparably lower	Does not required boiling of solid phase in IPE	Comparably higher	Employs boiling of solid phase in DEE to improve the separation efficiency
Scaling-up potential	Higher	Weighted conclusion	Lower	Weighted conclusion

The comparison is given for the processes with comparable separation efficiency

$BaCl_2$ barium chloride, *$Ba(OH)_2$* barium hydroxide, *CO* castor oil, *DEE* diethyl ether, *EtOH* ethanol, *FA* fatty acid, *FFA* free fatty acid, *HFAs* hydroxylated fatty acids, *IPE* isopropyl ether, *KOH* potassium hydroxide, *NHFAs* non-hydroxylated fatty acids, *RA* ricinoleic acid

[a] All descriptions and details on BVCI approach are given for purification algorithm described in Ref. [13], since this procedure ensures separation efficiency that is comparable to the developed process

[b] The data on the yield obtained with BVCI approach is unavailable in the literature, and consequently this aspect was not included in the present comparison

RA, without the utilization of costly technological approaches is feasible. Here, it should be acknowledged that the CCD method developed by Berdeaux et al. has the advantage of producing a relatively higher yield. The main differences among the developed approach and the previous processes employing salt-solubility-based fractionation of CO is summarized in Table 5.

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