Extraction of saffron ingredients and its fingerprinting by nano-emulsion membranes

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A novel approach has been introduced for the selective and tunable extraction as well as the fingerprinting of saffron solutes based upon the emulsion liquid membrane process. The objective of this method is optimizing the fingerprints, minimizing the solute variation, increasing the likelihood differences, obtaining the maximum extraction yield, and introducing the emulsion liquid membrane process in the fingerprinting extractions. The extractions are focused on the solutes of the interest including crocins, safranal and picrocrocin. Based upon the UV/Vis spectra in the range 230-530 nm, the component analysis of pre-processed data is conducted and the extraction parameters are optimized. The optimal conditions used for the best performance are surfactant’s type (Span 80), concentration (2.5 %), type of diluent (n-decane) in membrane, phase and treat ratios (0.8 and 0.3), and mixing speed (300 rpm). The repeatability of fingerprints is determined using surfactant 4 (2.5 %wt) and n-decane as the membrane phase.

Keywords: Crocin, Extraction, Nano-emulsion membranes, Safranal, Saffron

Saffron spice comes from the dried stigmas of *Crocus sativus* L. and its main use is as foodstuff. During the process of drying, the stigmas lose about 80 % of their weight and the moisture content of saffron reduces to 7-10 %wt.1. Crocetin, crocin (crocetin glycoside) and safranal are the major active constituents of saffron2.

Chemical analysis on *Crocus sativus* stigmas revealed that it contains carotenoids (crocetin), the glycosidic forms (crocin), gentiobioside, glucoside, gentioglucoside, diglucoside, γ-crocetin, trans-crocetin isomer, 13-cis-crocetin, α-carotene, β-carotene, lycopene, zeaxanthin and mangicrocin. Anthocyanins, flavonoids, vitamins, amino acids, proteins, starch, mineral matter, gums and other chemical compounds are also found to be presented in saffron3.

Among the constituents of saffron extract, crocetin is mainly responsible for its pharmacological activities4 like anti-alzheimer7, hypolipidemic activity8, anticonvulsant9,10 antipruritic and emmolient effects11 and antioxidant activity12.

Emulsion liquid membrane (ELM), invented by Li13, is known as one of the most promising separation methods for trace extraction of metal contaminants14-16 and molecular species17,18 owing to the high mass transfer rate, high selectivity, low solvent inventory and low equipment cost. Frankenfeld *et al*.19 reported that the ELM could be up to 40% cheaper than that of other routine solvent extraction methods. This process combines both extraction and stripping stages to perform a simultaneous purification and concentration. Some of the ELM's applications include the separation along with the concentration of phenol20, organic acids21,22, amino acids23-26, acetic acid27 and antibiotics28,29. Fig. 1 presents the structure of ELM system.

ELM process consists of a membrane phase, dispersed (or internal) phase, and a feed (or external) phase. Both dispersed and feed phases are aqueous solutions, whereas the membrane phase is a mildly hydrophobic organic liquid that forms a thin boundary layer with the dispersed phase. Normally, the feed aqueous phase contains the solute to be extracted. The membrane phase, being immiscible with both the

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dispersed and the feed phases, separates the two. This phase usually contains additives (carriers) and surfactants, which are chosen in such a way that the selectivity, permeability and stability of the membrane layer are enhanced. In a carrier-assisted ELM process, the membrane phase contains the complexing agent of carrier, which has a high affinity towards the solute of interest. This solute diffuses from feed phase to the feed:membrane interface, forms a complex (carrier:solute), permeates through the membrane and reaches the other membrane interface (internal:membrane), where the complex undergoes a chemical reaction to release the carrier agent into the membrane phase itself and another compound insoluble in the membrane but soluble in the internal phase. Thus, concentration of the solute at the internal:membrane interface is maintained at zero that allows a continuous driving force for the solute permeation through the membrane.

The most available techniques for this aim are solvent extraction\textsuperscript{30,31} and solid phase microextraction (SPME)\textsuperscript{32,33} methods. The main advantage of ELM with respect to other solvent extraction methods is more number of experimental conditions. Using ELM, it is possible to use more factors leading to have more components (PC1, PC2, PC3, etc). This condition increases the model validity. On the other hand, the previous solvent extraction methods use fewer factors. Moreover, another disadvantage of solvent extraction methods is their limitation to work at high speed of mixing (max 100 rpm) since two aqua phases are mixed, while it is not the case of ELM (300 rpm and more).

In this study, the ELM process has been used for fingerprinting the saffron extracts. The $p$H of the striping solution is chosen to be 11, while that of the treatment phase is 3. This difference in $p$Hs acts like a driving force to overcome to the equilibrium conditions. The species existing in the feed phase are present in the acidic form and the concentration of the basic forms is zero. The species existing in the strip phase are present in the basic form and the concentration of the acidic forms is zero. After penetration of acidic form of solute into the stripping phase, it is converted to the basic form. Hence, the concentration of acidic form is zero in the stripping phase. Likewise, there is not any tendency for carriers to transfer the basic form of solute from stripping phase into the feed phase. For this aim, different conditions of ELMs are assessed and the variations are used for classification. The spectra of UV/Vis are used in the wavelength range 230-530 nm, and component analysis of the pre-processed data is conducted.

**Experimental Procedure**

**Chemicals and instrumentation**

Surfactant sorbitan monooleate (Span 80) was purchased from Sorbitan Monooleate (Sigma Co.), ENJ-3029 was provided by Exxon Chemical Co. and polyamine-type polymeric surfactants [LMA (MW:9150) and Lan 113A] were gifted by Research Institute of Petroleum Industry (RIPI). $n$-Decane, toluene, chloroform and carbon tetrachloride were obtained from Sigma-Aldrich for use as diluent.

The UV/Vis measurements were carried out by spectrophotometer UV-1700 pham spec (Shimadzu, Japan) in the range 230-530 nm.

**ELM preparation**

The specific amount of surfactants was dissolved in the specific amount of $n$-Decane to prepare the membrane solution. Double distilled water was used as stripping solution ($p$H 10). In 10 mL beaker, stripping solution was added dropwise to the stirred membrane solution and the two-phase system was stirred continuously for 30 min at mixing speed of 1500 rpm by a variable speed mixer equipped with a turbine-type teflon impeller. The mixture of the membrane and the stripping solution was emulsified. The size, size distribution and stability of emulsions are characterized to examine the method. Size and size distribution of (w/o) droplets was obtained by optic microscopy.
(Mettler FP). The digital format of captured micrographs was analyzed by means of image analyzer software (Digital Micrograph TM, Gatan Inc.). Using a Neubauer camera, the volume of analyzed samples was controlled. By size distribution changes at constant times, the stability of w/o droplets was monitored and evaluated by image analyses from photographs obtained during the filtration experiments.

**Solutes extraction by ELMs**

Dried stigmas of saffron were milled, weighted and suspended into pure water. The solution was stirred continuously for 30 min and the suspensions were filtered. The temperature was kept below 10°C to reduce the volatile losses. The supernatant was used as the feed phase. In 5 mL vial, the ELM prepared was added to some volumes of the above-mentioned feed solution (pH 3) and were stirred by a variable speed mixer equipped with a turbine-type impeller at a speed of 500 rpm for extraction time of 15 min.

The extraction conditions were evaluated and the optimal conditions were obtained. The extractions were conducted at different extraction time (5, 10, 15, 20 min). The extraction efficiencies were increased from 5 min to 15 min. After that it remained constant. Therefore, the extraction time of 15 min was selected as the minimum time for the maximum extraction.

The speed of the mixer was regulated by a voltage regulator. After the time, the feed phase of the samples was separated from the emulsions by filtration using a filter paper. The emulsions were demulsified by the freezing. Then the supernatant was used for spectroscopic measurement.

**Statistical analyses**

Each spectrum was segmented into 300 spectral bars between 230 nm and 530 nm, corresponding to a bar width of 1 nm, using custom-written m-file in MATLAB. The areas within the spectral bars were integrated to yield a 1x300 vector containing absorption-based descriptors of the UV/Vis spectrum. The bars located in the ranges 250-300, 300-350, and 350-550 nm represent picrocrocin, safranal and crocins respectively. After that, to facilitate the comparison between the spectra, total spectral area of the remaining bars was normalized to unity. Then, the data bars were mean-centered. Principal component analysis (PCA) of the pre-processed data was conducted using the PLS Toolbox within the MATLAB. In this pattern recognition technique, the algorithm calculates the highest amount of correlated variation along the first principal component (PC1), with subsequent PCs containing correspondingly smaller amounts of variance.

**Results and Discussion**

**Effect of surfactant type**

The type of surfactant is one of the important factors that influences the selectivity of an inclusion-ELM system and can often be used in relevant extractions. The surfactant stabilizes the emulsion by reducing the interfacial tension and avoiding the coalescence of globules. In this study, four surfactants namely ENJ-3029 (1), ECA4360J (2), Lan 113A (3), and Span 80 (4) are studied. The effect of surfactant type on the extraction efficiency of solutes has been studied using ELM process and the spectral results obtained are shown in Fig. 2. According to the data,

![UV/Vis spectra of extracted solutes using surfactant 4 (230 nm < λ < 530 nm)](image)
there are three different peaks in the spectra for extracted analytes in the ELMs.

The criteria for evaluating the quality of an extraction method for fingerprinting include ease, yield, reproducibility and speed. Since the solutes are often co-extracted with other compounds, the net yield of extracts as the total peak height provide an approximate approach for comparing the extraction yields by different surfactants. Hence, the peaks height in each ELMs is normalized by the maximum height and the sample mass, and then the results are compared between the ELMs. Based upon the spectral data, surfactants 1-3 cause smaller yields, while the solutes are generally recovered to a greater extent by surfactant 4. Figure 3a represents the scores plots of UV/Vis spectra and shows distinct differences between the fingerprints of the extractions by different surfactants. Based upon the results, surfactant 1 tends to extract non-specific macromolecules with high molecular weights, such as carbohydrates. The corresponding spectra for these materials are presented on the negative side of Fig. 3a. The ellipses show mean (±SD) for each of extractions. By comparing the extraction procedures for surfactants 1-4, it is obvious that the repeatability of 4-mediated ELM is more than that of three others.

**Effect of membrane type**

Since the different liquid membranes have different polarities, it is observed that different solutes are extracted with differing ratios. As mentioned before, total UV/Vis spectral height of each extraction is normalized to sample mass and maximum height in order to compare yields between liquid membranes. According to the UV/Vis data, the yield of extractions using different liquid membranes is in the order: carbon tetrachloride ≈ chloroform ≈ toluene < n-decane. According to UV/Vis data, there are four different spectra for extracted solutes using different membranes of carbon tetrachloride, chloroform, toluene, and n-decane in the ELMs. Figure 3b depicts the scores plots of UV/Vis spectra and distinct differences between the fingerprints of the extractions by four types of membranes. According to the results, the chlorinated liquid membranes tend to extract the high molecular weight macromolecules. The corresponding spectra for these materials are presented on the negative side (Fig. 3b). By comparing the solute extractions using different membrane types, the repeatability is found to be in order: toluene < n-decane ≈ carbon tetrachloride ≈ chloroform.

**Effect of surfactant concentration**

Based upon the peak height, the extraction of saffron solutes increases by increasing the concentration of surfactant 4 from 0.5% to 2.5 %. Furthermore increase in surfactant concentration from 2.5% to 10% hardly affects the extraction performance; the efficiency of extraction is decreased owing to the access of molecular state in membrane phase (Fig. 4a).

Under the optimum concentration, the molecular form of surfactant 4 is considered enough to forward the extraction. Increasing surfactant 4 concentration up to 2.5% increases the stability of emulsion liquid membrane, which leads to the decrease in the break-up rate. Hence, the extraction of solutes increases. Further increase in the concentration of surfactant 4 leads to the decrease in the rate of capturing and stripping reaction. This is because the solutes remain in the membrane without being

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Fig. 3—Scores-plots of ELM + UV/Vis spectra using (a) surfactants 1-4 and (b) different membranes
stripped. This affects the final recovery by the ELM process.

The excessive surfactant 4 tends to increase the interface's resistance and increases the viscosity of membrane. This increase in surfactant concentration from 2.5% increases the emulsion stability but the mass transfer is adversely decreased. Hence, the optimum concentration of surfactant 4 is expected to be at around 2.5%. The excess of surfactant 4 concentration leads to osmotic swelling and membrane breakdown. Hence, the concentration of 2.5% is accepted as optimum concentration. Another criterion is the financial aspects, in which the surfactants are the most expensive agents among the other components of ELM process, and the lower concentrations are preferred.

**Effect of stirring rate**

The speed of mixing is a key factor in the rate of mass transfer through emulsion liquid membranes. The effect of stirring speed was investigated in the range 100–500 rpm in order to obtain optimal speed with effective extraction of solutes in the ELM process. Figure 4b presents the effect of stirring rate on the extraction efficiency. When the mixing speed is increased from 100 rpm to 300 rpm, an increase in extraction rate is observed. Above 300 rpm the extraction rate again reduces. As a result, an increase in the mixing speed would increase the interfacial area, and this is found to be true up to certain level of mixing speed beyond which an increase in the speed is likely to break the emulsions, thereby reducing overall enrichment and the efficiency of extraction. The impact on the wall of a contactor on the emulsion droplets or the shear-induced breakage of fragile emulsion droplets near the tip of the impeller imposes upper limit on the speed of agitation. At the same time, swelling is also increased owing to the transport of water from feed to strip phase. Some particles are broken owing to shear after reaching larger size. The swollen droplets are breakdown on their own or induced by shear. Therefore, the extraction performance is a trade-off between two effects of swelling phenomena and mixing speed.

**Effect of phase ratio**

The phase ratio is defined as the volume of stripping solution to volume of membrane. The effect of phase ratio on the extraction of solutes reveals that it increases with an increase in phase ratio up to 4:5. Fig. 5a presents the effect of phase ratio on the extraction efficiency. At 4:5 phase ratio, the maximum extractions are observed. By increasing the volume of the strip phase, the thickness of film in the emulsion is reduced, owing to the dispersion of strip phase in the membrane by mixing. This is favorable in extractions and results in an increase in the extraction of solutes. Beyond 4:5 phase ratio the further increase in the volume of strip phase causes the instability of globules.

**Effect of treat ratio**

The treatment ratio, defined as the volume ratio of the emulsion phase to the feed phase, plays an important role in determining the efficiency of ELM process. Figure 5b presents the effect of treatment ratio on the extraction efficiency. By increasing the amount of emulsion in the feed phase, the number of available droplets and interfacial surface area per unit volume of the feed solution increases. This leads to increase in the mass transfer of solutes from the feed to the membrane, and more efficiency. Increasing the treat ratio slightly increases the size of emulsion.
droplets and causes inversely a reduction in interfacial surface area. The increment in the size of droplets is suppressed by the increment in the number of droplets. The extraction efficiency is improved by increasing the treat ratio from 0.1 to 0.3. Beyond 0.3, the further increase in the ratio causes the instability of globules and less extraction efficiency.

The optimum conditions for the extraction of solutes are determined by the method “one-at-a-time”. Table 1 presents all the conditions tested as well as the optimum conditions in bold.

**Conclusion**

A novel ELM-UV/Vis method has been introduced to minimize the solute variation from analysis, and to improve the likelihood of solute differences and the extraction yield. Considering the yield of extraction and fingerprint, the operational processes of ELMs are optimized. Owing to the possibility of selecting different membranes, this method also benefits from extracting the hydrophobic and hydrophilic solutes into different fractions. The repeatability of fingerprints for 4-mediated ELM is observed more than using other surfactants. Different membrane types have been examined and the fingerprints repeatabilities are found to be in the order: toluene < n-decane ≈ carbon tetrachloride ≈ chloroform. Based on the UV/Vis data, the yield of solute extractions follows the order: carbon tetrachloride < chloroform < toluene < n-decane.

**References**

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