The “ouzo effect”, recent developments and application to therapeutic drug carrying

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Abstract. This short review is about the spontaneous emulsification effect, aka the “ouzo effect”. Under certain conditions, pouring a mixture of a totally water-miscible solvent and a hydrophobic oil into water, generates spontaneously nanometric droplets which are stable, even without surfactant. A basic example is anise-flavored aperitif, which is known from ages in South Europe and North Africa. Then, it is an amazingly old topic, potentially important in a number of applications – such as food additives, paints, cosmetic products or pharmaceutical drugs –, though the main mechanisms are yet essentially unexplained. This phenomenon is presently under intensive investigation using both microfluidic experiments and large-scale numerical simulations, through a CNRS project grouping four laboratories in France. This presentation will give an overview of the history, context and development of the ouzo effect, as well as recent advancements and ideas in the field. This unique effect is now related to two major streams of the scientific research, namely: nano-technology and bio-technology. Consequences in the latter domain is outlined.

1. Introduction

The “ouzo effect” [1] is a general method to emulsify various solutes (including polymers, oils, lipids, or pharmaceuticals) in aqueous media through a simple spontaneous emulsification route. The generality of the process makes it possible to produce large range of submicrometric particles, either solid or droplets. Moreover, by changing the solute content in the aqueous phase, as well as combining different solutes, it becomes possible to prepare nano-objects such as polymer capsules or fluorescent nanoparticles in a simple one-pot procedure. Maybe the best example to date is the technological breakthrough that the BASF Company accomplished by producing solid β-carotene dispersions that opened a new market for food additives in various dietary formulations [2].

Although the principle behind the ouzo effect has been known for a long time, many unsolved issues remain on the nature of the driving force that leads to metastable nanocolloids. In particular, the proper mechanisms occurring during the irreversible evolution to the metastable state are unclear and disagree with the classical theories of the nucleation.

We discuss below the state-of-the-art theoretical approaches of this phenomenon, and explain how a precise understanding of the ouzo effect could be important to generate new efficient forms of pharmaceutical drug-delivery.
2. The ouzo effect

Mixing two immiscible liquids results generally in phase separation and formation of two pure liquid phases. However, it is often desirable to obtain emulsion, that is a stable fine dispersion of one liquid into the other. This is of paramount importance for the chemical industry, especially in foods [3], paints and cosmetic products [4], but also for pharmaceutical drug delivery [5]. Indeed, stable drops protected with a shell, provide generally a safe way to carry active compounds in an aqueous fluid.

The ouzo effect is a special kind of emulsification process, which is currently under tremendous investigation because it does not require energy (thus interesting if one is concerned with cost issues) nor surfactant (concern with environmental issues). The process may have alternative names [6], such as: “spontaneous emulsification”, “solvent shifting”, “coacervation with addition of a nonsolvent”, “solvent displacement process” or “nanoprecipitation”, reflecting the successive discoveries of the same phenomenon by different teams, in different domains.

2.1. why the name: “ouzo effect”? 

The ouzo effect is known from ages in anise-flavored alcoholic beverages. In Greece, the drink is called ouzo – hence the name –, while it is called pastis in France, sambuca in Italy, raki in Turkey, and arak in Eastern Mediterranean countries. Actually, all these beverages are made of anethole oil dispersed in alcohol. Anethole is an essential oil extracted by distillation from various anise plants which grow naturally in the Mediterranean regions, as well as south-east Asia (China and Vietnam).

The common special feature of all these beverages is their unique behavior during dilution with water. Adding a large amount of water causes indeed the clear liquor to turn instantly into opaque translucent milky-white color. This phenomenon is known as “louching”, and it is the most spectacular feature of the physical process. It is due to the instantaneous formation of a number of small drops of about 1 µm diameter, thus strongly scattering visible light.

2.2. a quick-and-dirty description of the “ouzo effect”

We can give here a very schematic description of the ouzo effect taking the example of the ouzo: at the very beginning of the process, the system is a ternary liquid system, with anethole oil and alcohol dispersed in water. Oil is almost insoluble in water though it is soluble in alcohol, while alcohol is soluble in water. Then, oil tends to be surrounded with alcohol molecules, and alcohol molecules are at ease when surrounded with the water fluid. Then, it looks natural that the system separates in two phases: drops of oil+alcohol, dispersed in water. However, the things are not as simple as that elementary explanation. Particularly, it fails to explain the size and stability of the droplets. If strict thermodynamic equilibrium was expected, the final system state should be indeed multiphasic.

Only recently, in 2003, this effect has been analyzed in great details by Vitale and Katz [1], who gave the naming “ouzo effect” for the phenomenon (and we keep here this terminology). In their paper, the authors drew and commented in details a phase diagram of the ouzo effect. Actually, it was not the first time that a phase diagram was shown for this kind of emulsification process [7, 8], however, Vitale and Katz were the first ones to put clearly the problem of relevance and meaning of this phase diagram. After this seminal paper, more complete phase diagrams have been drawn [9]. This approach strongly suggests that there exists a generic scenario – still not fully understood – of the ouzo effect. In other words, this process is not an ‘accident’ found
with special compounds at special values of the parameters, but it is a new general process
which should appear in many applications in definite range of the physico-chemical parameters.

Moreover, it appeared that the process was known and used well before this time, without
synthetic idea about the generality of the phenomenon. For example, the term: “spontaneous
emulsification” was introduced by Ruschak and Miller [10] in 1972 about a study on the ouzo
effect in the ethanol-toluene-water system. Actually, a review of a number of examples of the
ouzo effect described in scientific papers up to 2005, was recently completed by Ganachaud and
Katz [6].

2.3. main features of the ouzo effect

The first and most natural question to ask is about the size of the solvent+solute drops in
the dispersing phase. Amazingly, the experimental size distributions are found to be remarkably
monodisperse [11]. However, one does not know for sure the reason why the drop-growing pro-
cess stops, and why the definite size [12]. The typical size is found to range from 100nm [13] to
a few micrometers in diameter, and it depends essentially on the ratio of excess solute to solvent
[14] (i.e. subtracting the solute which remains dissolved in the final solvent/water solution from
the initial amount of solute). In particular, it does not depend on stirring, pH, or ionic strength
[15]. Also, such an emulsion appears to be stable for months [1].

Presently, most of the investigation efforts to understand the spontaneous emulsification,
focus on the experimental phase diagram of ternary solutions as it is expected to give the key
to control the entire process. Some parts of the diagram are well known, specially the binodal
line [16], which separates the domain of parameters for which the equilibrated system is under a
single homogeneous phase, from the domain for which several phases appear. The binodal line is
found writing that the thermodynamic chemical potential of all solution components is equal in
each phase. Another line of interest is the spinodal line [16], which separates the domain where
the phase separation grows by nucleation, from the domain where the different pure phases
appear spontaneously. This line is found as the boundary of thermodynamic instability of a
solution to decomposition into multiple phases.

It has been shown clearly that the ouzo limit – which separates the domain where the system
experiences spontaneous emulsion from the domain where the system evolves by nucleation or
spinodal decomposition – is indeed different from both the spinodal line and the binodal line.
Then, the common belief is that the ouzo limit cannot be explained by classical thermodynamics
at equilibrium, but is directly related to the dynamics of appearance of metastable states. In
this context, the very definition of ‘phase diagram’ becomes ambiguous, as this diagram should,
in principle, be related to the only equilibrium states of the system. However, if we suppose
that the phase diagram is still meaningful for metastable states too (at least these metastable
states of very low energies), one can draw a general sketch as outlined on the figure 1.

2.4. Kinetic Monte-Carlo approach

Since classical thermodynamics requires the system at the equilibrium for the state variables
be properly defined, such an approach of the ouzo effect looks questionable. Indeed,
spontaneous emulsification process starts with strong off-equilibrium evolution, which makes
direct application of the free energy concept hard to justify. This may be a reason why the various
theoretical approaches of the ouzo effect is in quantitative disagreement with the experimental
data. Actually, any correct theoretical description of the phenomenon should take into account
the irreversible evolution of the system.
Figure 1. Generic phase diagram for solute-solvent-water ternary mixture, when solute is not soluble in water. The continuous line is the equilibrium binodal line that separates the single-phase region from the two-phase region. The dotted line is the spinodal line which separates the domain where the system evolves by nucleation (the domain with blue dots), and the domain where spontaneous phase separation occurs (the domain with yellow stripes). The ouzo phase (domain in pink color) is limited inside the region of the bi-phase equilibrium state, between the binodal and the spinodal lines. In this domain, the mixture decomposes into a monodisperse population of nanometric drops dispersed in an aqueous solution.

Alternative theoretical approaches requires numerics. Moreover, numerical simulations are particularly useful to analyze short-time behaviors. They can be Molecular Dynamics or Monte-Carlo methods.

Molecular Dynamics [17] has the advantage of being quite realistic [18]: if we know precisely the interactions between the different species, one solves simultaneously all the equations of motions to find the complete evolution of the system. Of course, one has to pay the accuracy of the method with a huge amount of calculations necessary to realize a simulation. Then, the Molecular Dynamics method is restricted to short times and small systems.

The Kinetic Monte-Carlo method [19] does not need so serious computation cost. One can draw here the main lines for the specific case of the mixing of several liquids [20]. The system is approximated as an ensemble of molecules on the sites of a regular lattice. Then, each site has the same number, say $z$, of neighboring sites. The cubic 3D lattice is the most popular example, though use of other lattices can give more realistic visual impression.

2.4.1. exchange energy between two neighboring molecules

As we are dealing with a continuous fluid, all the sites of the lattice must be occupied with molecules. Moreover, a lattice site can be filled with one and only one molecule at a given time (steric exclusion). It means that one assumes that the molecules of different species are all of about the same volume. Moreover, neighboring molecules are assumed to be linked together with chemical bonds. The bond energy between a molecule $A$ and a molecule $B$, is noted $E_{AB}$. We have then to estimate the difference of energy of the system when two molecules $A$ and $B$ exchange their positions.

A key remark is that all the changes of energy appear under combinations of the only reduced
exchange energies:

\[ W_{ij} = \frac{z}{k_B T} \left( E_{ij} - E_{ii} + E_{jj} \right) \]  

(1)
in which \( z \) is the number of neighbors of a molecule (on the cubic lattice: \( z = 6 \), which is a reasonable value for real molecules), \( k_B \) is the Boltzmann constant \((k_B = 1.38 \times 10^{-23} \text{ J/K})\) and \( T \) the temperature in K. Indeed, suppose that the couple of neighboring molecules \( A \) and \( B \) undergoes position exchange. Let us denote \( i_k \) the sites neighbors to \( A \) and \( j_k \) the sites neighbors to \( B \), before position exchange. Then, the change, \( \Delta E \), of the system energy during the move, writes:

\[ \frac{\Delta E}{k_B T} = \frac{1}{k_B T} \sum_{k=1}^{z} \left( E_{Bi_k} + E_{Aj_k} - E_{Ai_k} - E_{Bj_k} \right) \]

\[ = \frac{1}{z} \sum_{k=1}^{z} \left( W_{Bi_k} + W_{Aj_k} - W_{Ai_k} - W_{Bj_k} \right) \]

Generally, values of the non-dimensional \( W_{ij} \) are positive as a consequence of Berthelot’s geometric mean of the constants of the pure components \([21]\). For the ternary system made of three sorts of molecules \( A, B, C \), one has to consider three exchange energies, namely: \( W_{AB}, W_{AC}, W_{BC} \), and we have not to consider the exchange of molecules of the same sort (exchange of two neighboring molecules \( A \), for example, does not change the state of the system).

2.4.2. Probability per unit of time to exchange two neighboring molecules

A characteristic time, say \( \tau_e \) is attached to the microscopic position exchange of two neighboring molecules. According to the Metropolis algorithm \([22]\), the probability, \( P_e(A, B) \), per unit of time for two molecules \( A \) and \( B \), to exchange their position, is then:

\[ P_e(A, B) = \frac{1}{\tau_e} \text{ if } \Delta E \leq 0 \]

\[ P_e(A, B) = \frac{1}{\tau_e} e^{-\Delta E / k_B T} \text{ if } \Delta E > 0 \]  

(2)

where \( \Delta E \) is the amount of energy required for the move. The probability of the reverse event (the exchange \((B, A) \rightarrow (A, B)\), all the rest of the system being identical), is such that the detailed balance condition:

\[ P_e(A, B) = e^{-\Delta E / k_B T} P_e(B, A) \]

is realized.

2.4.3. The Brownian motion of the clusters

A cluster is defined as an ensemble of molecules of the same sort, connected together through continuous sequence of neighboring bonds. The ability of the lattice approximation to define simply the clusters is a great advantage of the Monte-Carlo approach (in the Molecular Dynamics method, the proper definition of the clusters is not that clear). Then, one can consider the Brownian translation and Brownian rotation of the clusters.

Brownian translation event by a lattice step occurs with the characteristic time \( \tau_t \) depending on the size of the cluster and the viscosity of the liquid. Here we will choose a simple formula
such as the Stokes formula: $\tau_t = \tau_0 R_\perp$, where $R_\perp$ is a typical cluster radius perpendicular to the move, and $\tau_0$ the characteristic time for the Brownian motion of a single molecule. The liquid viscosity is supposed to be uniform throughout the system. Use of the Stokes drag formula is indeed a crude approximation. It requires in principle that the diffusing molecule keeps a compact shape, and that its own velocity is small. If we could define the Reynolds number at this scale, it should be of order $10^{-3}$ in water, which is a small number, then the Stokes drag formula is probably a good approximation.

Then, we define the probability, $P_t(C)$ of translation by a lattice step, of the cluster $C$, in a way consistent with the notations of the previous section, that is:

$$P_t(C) = \frac{1}{\tau_0 R_\perp}$$

(3)

and the translation occurs along one of the $z$ possible directions allowed by the lattice structure. Note that the larger the cluster, the smaller its translational probability per unit of time. During the move, molecules of the fluid not belonging to the cluster, have to be displaced. A random rule mixing is used, that is these molecules are put randomly in the sites made vacant by the cluster move.

The case of the rotational motion is more difficult to handle on a lattice because of the geometric constraints. Indeed, a cluster can rotate by a finite angle (e.g.: $\pi/2$ on the cubic lattice). As we consider only small displacements, the point around which the cluster rotates is chosen randomly among the sites of the cluster. The probability of rotation per unit of time is chosen similarly to the translational move, that is:

$$P_r(C) = \frac{1}{\tau_0 R_\perp}$$

(4)

where $R_\perp$ is the value of the cluster radius perpendicular to the effective rotation, and $\tau_0$ a typical rotation time for a single molecule. Here too, random mixing rule is used for the displaced fluid.

2.4.4. the algorithm

First, we have total freedom on the initial configuration. The two most popular initial states are : the complete mixed system, where the molecules $A, B, C$ are put randomly on the lattice ; or the multiphase system, where the initial system is divided into two or more homogeneous phases (a unique cluster of $A$ molecules in a homogeneous mixed fluid of $B$ and $C$ molecules, for example). Both initial states correspond clearly to two different initial mixing ways.

The definite algorithm is as follows :

- at a given time, all the possible elementary movements (exchange of two neighboring molecules, translations or rotations of clusters) are considered with their corresponding probabilities per unit of time;
- the sum, $\Sigma$, of all probabilities is computed and the list of probabilities is normalized by this sum. That way, the sum of all normalized probabilities takes the value 1 (and this corresponds to time re-scaling) ;
- then, one event is selected in that list according to its probability. This event is realized, and the physical time is implemented by the amount $\log(1/X)/\Sigma$, with $X$ a random number uniformly distributed on $[0, 1]$ (to insure that the increment of time is Poisson-distributed).
The system evolves according to these simple probabilistic rules and is able to find naturally metastable states in the canonical ensemble. As we know that the ouzo phase is metastable, the Kinetic Monte-Carlo method is preferred in the context. A pictorial example is shown on the figures 2, 3 and 4, where three states of a same 2D ternary system on the hexagonal lattice, at three different times, are shown. The system size is $100 \times 100$ lattice sites, that is a small size which has been chosen only for clear visualization (sizes of $1000 \times 1000 \times 1000$ on the 3D lattice can be reached easily). The values of the exchange energies were respectively: $W_{AB} = 2.5$ which corresponds to a limited solubility of $A$ in $B$ (roughly speaking, exchange energy value $< 2$ means that the two substances are soluble one in the other, while a value $> 2$ means insolubility); $W_{AC} = 7$, that is $A$ is insoluble in $C$; $W_{BC} = 1.2$ that is $B$ is soluble in $C$.

It is clear on the figures that the system tends to form clusters of molecules $A$ surrounded by cloud of molecules $B$ in the fluid $C$. Then, due to attraction of the molecules $B$ for $C$, two clouds of molecules $B$ are seldom to merge, that stabilizes the already formed structures of $A$-clusters with $B$ shell. This is consistent with the remark that no drop coalescence is observed in experimental ouzo process [23].

![Figure 2](image1.png) **Figure 2.** Totally mixed initial state of a 2D ternary system on the hexagonal lattice. The volume fractions are respectively: 2.5% of molecules $A$ (yellow circles); 5% of molecules $B$ (green circles); 92.5% of molecules $C$ (the black part).

![Figure 3](image2.png) **Figure 3.** At a later time, the system begins to self-organize in small clusters of molecules $A$ (yellow), because they are essentially insoluble in the fluid $C$ (black). The system evolution is irreversible at this stage, and the total energy is decreasing with the time.

![Figure 4](image3.png) **Figure 4.** The steady state is made of clusters of molecules $A$ (yellow) surrounded with molecules $B$ (green). That way, the system acquires a small total energy in a metastable phase, even if the strict minimum energy should be for separated phases.

2.5. The importance of microfluidic devices

Microfluidic devices [24] have been shown to be able to control finely patterned self-organization [25] or crystallization [26] at the microscopic scale. This is a promise candidate for obtaining high-quality nanoparticles. Also, a number of works using microfluidic, were devoted to the general formation of emulsion [27, 28]. It is then interesting to use this technique to investigate the mechanisms acting in the early stages of the self-emulsification effect. The general concept of such experiments is sketched on the figure 5.

Indeed, as the process is highly irreversible at the beginning, the short time history of the evolution is of prime importance to understand the spontaneous appearance of the metastable
Figure 5. Schematic representation of the microfluidic “Y-junction” used in LOF laboratory, Pessac, France (J.-B. Salmon) to study the ouzo effect. Typical microfluidic geometries are \((h\) and \(w\) are defined on the left-hand figure): \(h = 10 \sim 50 \mu m, w = 10 \sim 1000 \mu m\). Water and solvent+solute are introduced through converging channels, meet at the junction and flow out through the exit channel. Interdiffusion takes place in the laminar exit stream. The distance downstream in the channel is related to the time elapsed since the two streams were put into contact: the distance \(x\) from the junction corresponds to the outcome of interdiffusion-reaction over a time \(t = x/v\) (\(v\) is the average velocity in the channel). It is thus possible to analyze in details the very beginning of the ouzo process with specific analytical tools at time scales down to several tens of ms \((v \simeq 1 \text{ cm/s}, x \simeq 100 \mu m)\), and to compare with numerical simulations in the same geometry.

phase. Moreover, the short times are accessible to the Kinetic Monte-Carlo method, then direct comparison between real experiments and numerical experiments with the same geometry and time range, is possible. This work is presently in progress.

3. A short introduction to medication delivery

Now, we would like to finish with possible application of the ouzo effect in an important field: pharmaceutical drug-delivery.

In most of the cases, active ingredient of a drug must reach special inner place in the body of a patient. The target can be diseased tissues – such as malignant cells –, or virus-infected cells. The fastest and most direct way to act, is to use parenteral injection in the circulatory system. This way avoids indeed first-pass metabolism, that is the sudden decrease of drug concentration occurring in the digestive system. However, severe conditions must be fulfilled to insure the intravenous therapy be safe. Here we will focus on two important aspects: the medication has to be a liquid solution or a fine dispersion in a liquid solvent; the solvent must not react with blood and blood vessel, from the double point of view of the chemistry (chemical inactivity) and physics (isotonic liquid).

3.1. the liposomes

Using a homogeneous liquid solution is the simplest way, but there are immediate drawbacks: the liquid active chemical compound may interact with any cell irrigated with the blood. It results in possible disorder due to uncontrolled toxic interaction.

Alternatively, a very active research domain in the field pertains to preparation of dispersions of nanometric particles in isotonic solvent [29]. The active ingredient is then trapped inside the nanometric particles, and it is released when the target is reached. The most popular particles – discovered fifty years ago –, are the liposomes [30]. A liposome is a closed vesicle of lipid bilayers
which encapsulates small amount of aqueous solution in which the active ingredient is dissolved. As water cannot pass through the lipid layers, the aqueous solution is permanently trapped in this small bag, until the vesicle opens. From the point of view of physics, the system is then a mixture of two liquid phases (the blood as the continuous phase, and the aqueous solution with the active drug as the dispersed phase), which are separated one from the other by lipid interface.

The protective layer is treated such that the immune system does not destroy the vesicle for a long time in the bloodstream, typically for a day or more. The stealth character is obtained studding particular chemical compound (such as polyethylene glycol) on the surface of the outer lipid layer, making it inert in the circulatory system and in the cleansing organs. On the other hand, a chemical mechanism breaks up the layer when the liposome is close to its target. This is achieved grafting special peptides on the surface of the liposome. Indeed, the peptides bind specifically to special receptors present on the target cells, then, once bound to a receptor, the lipid layer becomes weak and opens, releasing the tiny amount of active ingredient in a concentrated way at the right place.

Even if lipid vesicles are used nowadays to carry a number of pharmaceutical payloads, their formation remains generally very delicate, while the efficiency depends dramatically on characteristics hard to control, such as: polydispersity, shelf-life, and batch-to-batch reproducibility. Actually, liposomes are not generated spontaneously, and one needs input of energy to produce them. This is generally achieved by high-energy sonication of natural or synthetic phospholipids in the medication solution [31]. However, the quality of the resulting liposomes is poorly controlled using this method. More sophisticated methods were contrived, but they all encounter the problem of the small production rate. At the end, the difficulty for obtaining reliable and efficient manufacturing techniques, results in high production costs which still limit wide applicability of the method.

3.2. the alternative use of spontaneous emulsions

A liposome dispersion is a particular case of nanometric emulsion. However, up to now, ordinary emulsion (with addition of surfactant to stabilize the droplets) could not be used for intravenous injection because the usual surfactants easily destroy the blood cell walls. Then, the ouzo effect appears to be the perfect candidate to manufacture monodisperse droplets of therapeutic drug in aqueous medium compatible with blood. Moreover, the typical sizes for the droplets appearing by the ouzo effect are comparable to the optimum efficient sizes (around 100 to 500 nm) to destroy the cancer cells [32]. Then, as soon as we shall understand how the phase diagram of the ouzo effect is build and is structured according to the solute compound, such a biotechnology will be at our fingertips.

4. Conclusion

We have seen in this review that the ouzo effect is a versatile way of forming nanometric droplets of a liquid encapsulated in a protective shell. Such a process is attractive: it is spontaneous, mild, reproducible and gives rise to controlled monodisperse emulsion. The only serious flaw is that we do not understand yet the mechanisms leading to the self-organized systems. Without such a knowledge, one can only proceed by “trial-and-error” to find the emulsification operating window. In 2011, a three-years CNRS project started in France to elucidate the ouzo effect. This project is expected to give soon the keys to understand, thus to control, the spontaneous nanoprecipitation process.
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