



# The nanostructure character of Arnica Montana as ultra-high diluted succussed solution medicinal product. Recent advances and prospects

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**Abstract**

**Objective:** Arnica Montana is a very useful and widely used medicinal plant by established, traditional, complementary and Alternative Medicine. This manuscript describes the physicochemical evaluation of ultra high diluted-succussed solution products based on Arnica Montana (starting material) as they are used like remedies by TM/CAM (Homeopathy).

**Methods:** Scientific methods of analysis like SEM, XRD, FTIR, micro Mastersizer, nano Zetasizer, pH-meter, Electrical Conductivity meter and TEM showed that the process of preparation of these products (trituration, dilution and succussion) changes the physical characteristics and the constituent profile of the initial substance. This, of course, is due to the micro-nano character of the final products.

**Results:** The findings of this study showed that even in the last ultra-high diluted-succussed solutions, the starting materials undergo changes in their physicochemical properties as they are formed at the end of the trituration process.

**Conclusion:** In this paradigm, the conclusion is that the whole preparation process leads to the creation of micro-nanoparticles, which are encountered in all ultra-high diluted-succussed solution products.

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**Keywords:** Arnica; Product; Nanoscale; Nanostructure; Nanoparticles; Nanoremedies

**Abbreviations:** AM: Arnica Montana; TM/CAM: Traditional Medicine/Complementary Alternative Medicine; RSM: Raw Starting Materials; SEM: Scanning Electron Microscope; XRPD: X-Ray Powder Diffraction; FT-IR: Fourier Transform Infrared Spectroscopy; EDL: Electrochemical Double Layer; TEM: Transmission Electron Microscopy; DLS: Dynamic Light Scattering; ZP: Zeta Potential; NP: Nano Particles; Pdi: Polydispersed index; UH-DSSP: Ultra High Diluted Succussed Solution Products.

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## Introduction

AM is a medicinal plant widely used as herbal remedy as well as in cosmetics industry. Different plant parts such as inflorescences, rhizomes, roots and leaves are collected for healing purposes. Selected constituents contained in raw materials are volatile oils, terpenoids, sesquiterpenes lactones, flavonoids, coumarins, carbohydrates resins, tannins, carotenoids. These substances show antiseptic, anti-inflammatory, inotropic, antibiotic, analgetic, antibacterial, antifungal, antioxidant and anti-tumor activity [1-9]. The most active components are sesquiterpene lactones, which are known to reduce inflammation and decrease pain, and show antioxidant and cytotoxic effects. AM contains some constituents that may change platelet function and can lead to interactions with anticoagulants [10]. AM is also mentioned as a good first aid salve for sport injuries and discomforts. In TM/CAM specifically, it is used as the main treatment for trauma [11-15]. More than 100 drug preparations contain this plant as their main ingredient [16]. It is used by professional athletes to soothe sore muscles, by prominent cosmetic surgeons and dentists to relieve post-procedure pain, and by well-informed young mothers to treat playground bumps and bruises. Different bioactive secondary metabolites (sesquiterpene lactones, acids, oligosaccharides, oligofructosides, etc.) of AM have been investigated, while data on AM volatile oils are very limited, despite the fact that essential oils of the plant contribute to all plant medicinal properties [17-22]. Today it is used both herbally in the form of creams and tinctures as well as in the form of UHDSSP. The pharmacology of AM and its applications in established - traditional - complementary and alternative medical and dental practices has not been completely investigated, and thus it is intended for UHDSSP [23]. Usually, AM is used as UHDSSP made from extracted mother tinctures or from dried root's powder triturated in lactose [12]. This study was therefore designed to investigate the impact of the preparation process of AM, and specifically the particles size morphology during trituration before and after turning them into UHDSSP, any changes in the crystalline form or any chemical changes during mechanical grinding, the size dimension of the ingredients in the high ultra-diluted solutions, their electrical conductivity and pH. Then, the physical-chemical properties of superfine and crude AM particles are compared.

## Materials and methods

The materials used were:

- The dry powdered roots of AM plant origin in well closed bottle (AM powdered dry roots 5 g, 100% natural, from Organic Agriculture seeds), M and N Fillianos' Pharmacy benefaction supply.
- Distilled water filtered through a 200 nm syringe filter.
- Ethanol absolute denature with 1 % MEK and 0.001 % Bitrex GR for analysis 64271 Darmstadt Germany.

- Commercially manufactured crystalline  $\alpha$ -lactose monohydrate of different particles sizes was obtained from DFM Pharma GmbH & Co, KG, Gosh, Germany origin for pharmaceutical use.

The preparation process from initial to final triturated solid samples, as well as the required equipment were the same as the ones described in our previous work [24,25]. In addition, XRD (BRUKER D8 Advance), FT-IR spectrometer (FTIR Jasco 4200 with ATR PRO 410-S, TGS detector), pH-meter ConSORT C532 version 2 and Electrical Conductivity meter HACH Sension 7  $\mu$ S/cm were used.

To prepare solutions from initial substance up to the final solid sample, intended for measurements with laboratory equipment, 3 g (parts) substance from the previous triturated potency was diluted in 97 mL (parts) of distilled filtered water and ethanol 62 per cent V/V.

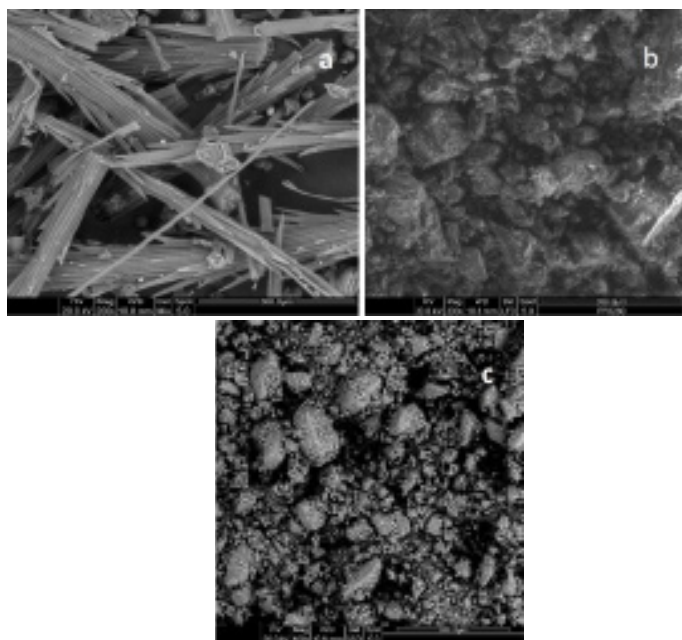
From the finale 6X liquid potency, 1 mL (part) of the 3C centesimal dilution and 99 mL (parts) of distilled filtered water were used to prepare the 4C dilution. Subsequent dilutions as the 12C, 30C, 200C, 1M, 10M, 50M are produced in the same way i.e. 1 mL (part) of the previous centesimal dilution and 99 mL (parts) of distilled filtered water. All the solid samples are examined by SEM, XRD, FTIR, micro Mastersizer and the liquid ones by nano Zetasizer, pH-meter, Electrical Conductivity meter and TEM. Thus, the effect of handmade trituration upon the AM's RSM, before turning these into UHDSSP is investigated i.e. if some physical properties varied during the process, as well as, in what form this material could be in the solutions. All measurements were performed in triplicate and the average value was considerate.

## Results and discussion

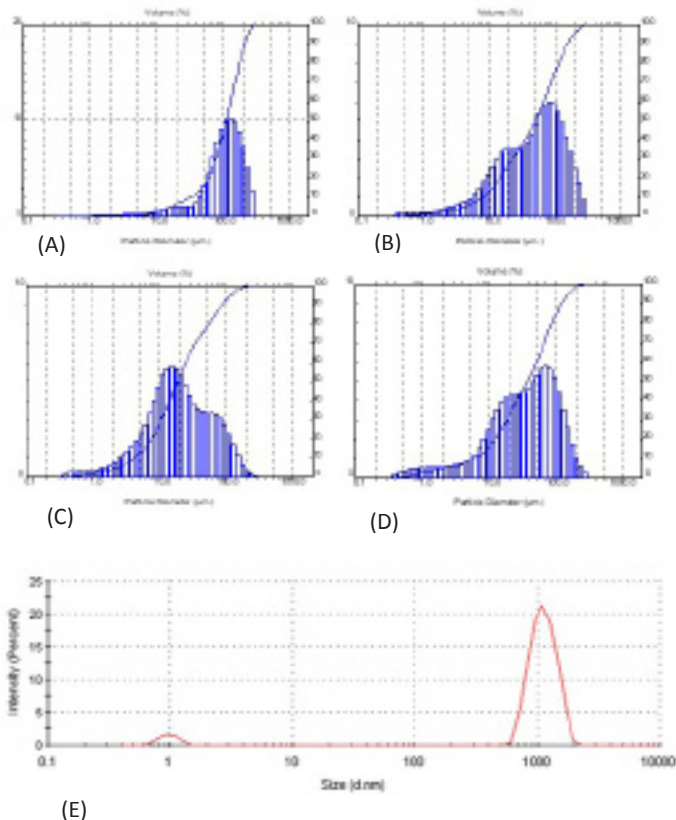
During this study, a number of instrumental methods of analysis have been used in order to investigate the changes in the physicochemical properties of AM from the beginning (RSM) up to last UHDSSP and the obtained results are presented. These changes are considered to be related to the gradual change in the size of the material during trituration.

### SEM - Master sizer granulometry analysis

The morphology of triturated AM was studied by SEM. The analysis revealed that the fibrous nature of AM particles was not further observed in the final (6X) triturated AM sample, where agglomerated AM particles in the micro size were undoubtedly shown on lactose (Figure1c). Also, the Master Sizer Granulometry Analysis is analogous as described in a previous work [25]. The triturated samples (initial, 1X, 2X and 3 X) were subjected to granulometry measurements and the results showed the change of the average size of the material's initial granules up to 3X trituration in the micro scale (Figure 2).



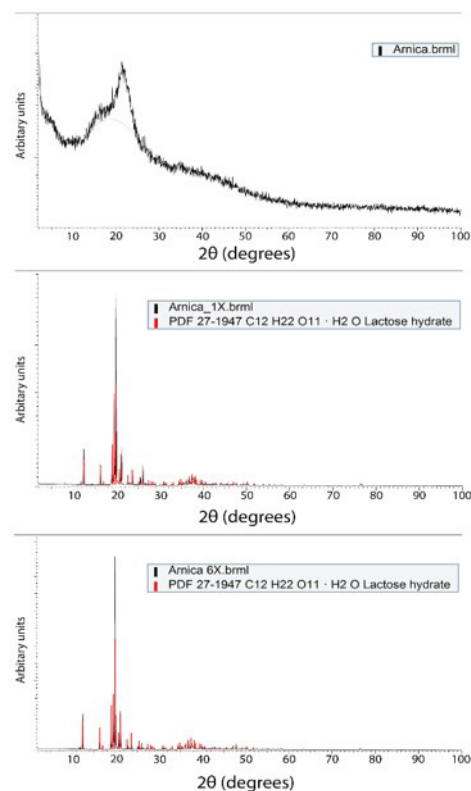
**Figure 1:** SEM images of Arnica Montana: (a) Source material, (b) 3X trituration in  $\alpha$ -lactose monohydrate, (c) 6X trituration in  $\alpha$ -lactose monohydrate.



**Figure 2:** Arnica Montana DLS-Master Sizer Granulometry: (a) Initial source material, (b) 1X trituration potency, (c) 2X trituration potency, (d) 3X trituration potency, (e) DLS-Nano Sizer Granulometry for 6X trituration potency.

### XRD analysis

A crystallographic RSM investigation was performed with XRD for initial, first up to last trituration potencies and the results are shown in Figure 3.



**Figure 3:** XRD spectra of Initial, 1X, 6X of Arnica's Montana samples

Analysis of the XRD patterns shows that the RSM of AM is mainly amorphous and there is almost a complete lack of crystallinity due to the nature of the sample (mostly cellulose). The broad peak near at  $22^{\circ}$ - $23^{\circ}$  indicates the existence of some crystalline substance, probably a salt. The other two XRD patterns, first (1X) and last (6X) present perfectly crystallized structures, expressed by the well-formed narrow and high in intensity peaks at  $20^{\circ}$ . All the red peaks correspond to  $\alpha$ -lactose monohydrate which presents a high degree of crystallinity.

### FT-IR analysis

Infrared spectroscopy has been used for gathering information on the chemical structure and functional groups of initial, first (1X) and last (6X) trituration solid samples (see supplementary materials). The detected vibrational bands were identified, and the corresponding functional groups are presented in Table 1. The key is to show which are the changes that chemical groups undergo during trituration process observing the final trituration solid potency, before it is turned into a solution and becomes mother tincture of other UHDSPP. The exact composition of the root initial sample material is not completely known. By comparing the spectrum of the initial with those of first (1X) and last (6X) trituration samples, it is observed that there are significant variations, as it is expected due to the presence of  $\alpha$ -lactose monohydrate in the first and the last samples. However, it is supposed/expected that all functional groups that correspond to the vibrational bands of the initial sample also exist in the other next samples. Although, the groups of amine, N-H and aldehyde, C=O, are not present in the first (1X) and last (6X) trituration sample. On the contrary, into the first trituration sample (1X), nitro group N-O emerges, which does not exist in  $\alpha$ -lactose monohydrate ( $C_{12}H_{22}O_{11} \cdot H_2O$ ). At the last trituration sample (6X), a vibrational band corresponding to the Alkynes Monosubstituted Disubstituted, Amines- Salts, primary RCN Aliphatic Nitriles, appears, in addition to the the nitro N-O group. In the spectra

presented, the bands at 3798.12, 3718.08 and 3749.9  $\text{cm}^{-1}$  are due to O-H bond type, which may be attributed to adsorbed humidity. The wavenumber 3970  $\text{cm}^{-1}$  belongs to Methyl-band C-H stretch [26]. Band of the hydrocarbons, because of methylene twisting and wagging vibrations, is observed in 1350-1150  $\text{cm}^{-1}$ . A series of bands in this region arising from the methylene group is a characteristic of the spectra of solid samples of long chain acids, amides and esters. Also, at 1340-1400  $\text{cm}^{-1}$ , there are overlapping doublets for t-butyl and isopropyl groups. The C-H stretching band of monosubstituted alkynes occurs in the general region of 3333-3267  $\text{cm}^{-1}$ . This is a strong band of C-H and is narrower than the hydrogen bonded OH and NH bands occurring in the same region. The FTIR spectra of plant-extract dilution suggest a very strong interaction of solvents (water in

this case) with chemical groups resulting in a structural rearrangement in the aromatic backbone. This is evidenced by the changes in the band positions, appearance and disappearance of bands. The diluted concentration of AM shows vibrational modes that resemble sesquiterpene lactone. This suggests that the process of serial dilution assists in changing the chemical environment in the backbone matrix of AM, thereby showing structural resemblance to a purified active derivative: helenalin, and dihydrohelenalin [27]. FT-IR analysis of pristine and triturated AM showed that during the basic process of Pharmacopoeia, apart from the reduction of grains size, there is an appearance of new compounds (existence of new vibration bands in the spectrum) in the mixtures that did not exist in the initial sample, indicating that in the case of AM, the trituration leads to a kind of mechanochemistry.

**Table 1:** FTIR band assignment for different samples of Arnica

Initial Characteristic Absorptions ( $\text{cm}^{-1}$ )	Functional Group Type of Vibration Intensity	1X Characteristic Absorptions ( $\text{cm}^{-1}$ )	Functional Group Type of Vibration Intensity	6X Characteristic Absorptions ( $\text{cm}^{-1}$ )	Functional Group Type of Vibration Intensity
3981.07	Methyl-band C-H Stretch	3904.18	Methyl-band C-H Stretch	3807.76	Methyl-band C-H Stretch
3899.36	Methyl-band C-H stretch	3718.08	O-H bond type	3749.9	O-H bond type
3798.12	O-H bond type	3650.59	-CH <sub>2</sub> -O-H O-H Alcohol-Phenol Free, Stretch- Strong, Sharp	3653.48	-CH <sub>2</sub> -O-H O-H Alcohol-Phenol Free, Stretch Strong, Sharp
3345.89	O-H Alcohol-Phenol Stretch, H-bonded Strong, Broad N-H Amines, Stretch medium (primary amines have two bands; secondary have one band, often very weak) N-H Amides Stretch Unsubstituted have two bands	3526.2	O-H Alcohol-Phenol Stretch, H-bonded Strong, Broad O-H Alcohol-Phenol Free Stretch Strong, Sharp	3614.91	O-H Alcohol-Phenol Free, Stretch, Strong, Sharp
2922.59	C-H Alkane Stretch Strong, O-H Acid Stretch Strong, Very broad	3330.46	O-H Alcohol-Phenol Stretch, H-bonded Strong, Broad N-H Amine- Stretch Medium (primary amines have two bands; secondary have one band, often very weak)	3331.43	O-H Alcohol-Phenol Stretch, H-bonded Strong, Broad N-H Amine- Stretch medium (primary amines have two bands; secondary have one band, often very weak)
2855.1	C-H Alkane- Stretch Strong, O-H Acid Stretch Strong, Very broad	2900.41	C-H Alkane- Stretch Strong, O-H Acid Stretch Strong, Very broad	2903.31	C-H Alkane- Stretch Strong, O-H Acid Stretch Strong, Very broad

1729.83	<p>C=O Carbonyl Stretch strong</p> <p><b>C=O</b> <b>Aldehyde</b> <b>Stretch strong</b></p>	1651.73	<p>C=C Alkene Stretch Variable C=O Carbonyl Stretch Strong, C=O Amide Stretch Strong,</p>	2320.91	<p><math>-C-C \equiv C-C-</math> <math>H-C \equiv C-</math></p> <p><b>Alkynes Monosubstituted Disubstituted</b></p> <p><b>Amines- Salts Primary</b></p> <p><b>RCN</b></p> <p><b>Nitriles Aliphatic</b></p>
1643.05	<p>C=C Alkene Stretch Variable</p> <p>C=O Amide Stretch Strong medium-weak, multiple bands</p>	1541.81	<p>C=C Aromatic Stretch medium-weak, multiple bands</p> <p><b>N-O</b> <b>Nitro</b> <b>Stretch Strong, two bands</b></p>	1652.7	<p>C=C Alkene Stretch Variable C=O Amide Stretch, Strong</p>
1599.66	<p>C=C Aromatic Stretch medium-weak, multiple bands</p> <p><b>N-H</b> <b>Amide</b> <b>Bending</b></p>	1427.07	<p>-C-H Alkane Bending Variable C=C Aromatic Stretch medium-weak, multiple bands</p>	1517.7	<p>C=C Aromatic Stretch medium-weak, multiple bands</p> <p><b>N-O</b> <b>Nitro</b> <b>Stretch strong, two bands</b></p>
1421.28	<p>-C-H Alkane Bending Variable C=C Aromatic Stretch medium-weak, multiple bands</p>	1336.43	<p>C-N Amine Stretch medium-weak</p>	1426.1	<p>-C-H Alkane bending variable C=C Aromatic Stretch medium-weak, multiple bands</p>
1253.5	<p>C-N Amine Stretch medium-weak C-O Ether Stretch Strong C-O Acid Stretch Strong C-O Ester Stretch Strong two bands or more</p>	1264.11	<p>C-N Amine Stretch medium-weak Ether C-O Stretch Strong C-O Acid Stretch Strong Ester C-O Stretch Strong two bands or more</p>	1348.96	<p>C-N Amine Stretch medium-weak <b>N-O</b> <b>Nitro</b> <b>Stretch strong, two bands</b></p>

888.059	=C-H Alkene Bending Strong	1130.08	C-O Alcohol Stretch Strong C-N Amine Stretch medium-weak Ether C-O Stretch Strong C-O Ester Stretch Strong two bands or more	1263.15	C-N Amine Stretch medium-weak Ether C-O Stretch Strong C-O Acid Stretch Strong Ester C-O Stretch Strong two bands or more
815.742	=C-H Alkene Bending Strong	987.375	=C-H Alkene Bending Strong	1142.62	C-O Alcohol Stretch Strong Amine C-N stretch medium-weak C-O Ether Stretch Strong C-O Ester Stretch Strong two bands or more
772.351	=C-H Alkene Bending Strong	900.584	=C-H Alkene Bending Strong	1065.48	C-O Alcohol Stretch Strong Ester C-O Stretch Strong two bands or more
		872.831	=C-H Alkene Bending Strong	1031.73	C-O Ether Stretch Strong C-O Ester Stretch Strong two bands or more
		771.387	=C-H Alkene Bending Strong	988.339	=C-H Alkene Bending Strong
				902.523	=C-H Alkene Bending Strong

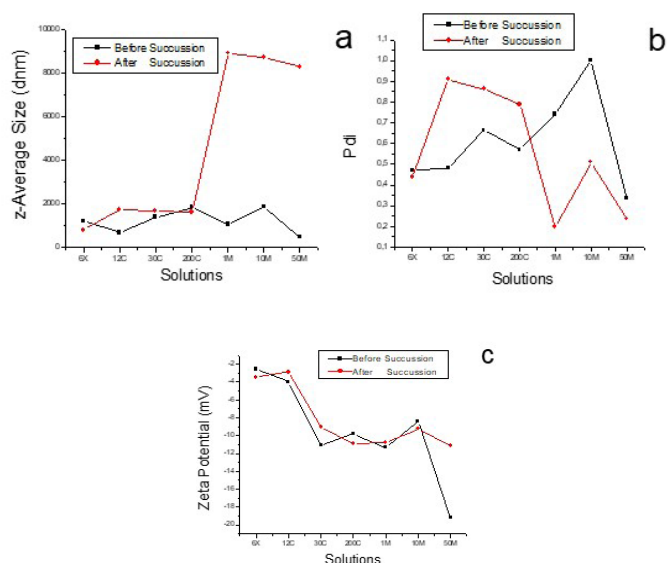
				868.774	=C-H Alkene Bending Strong
				772.35 (660-900)	=C-H Alkene Bending Strong

**NANO sizer granulometry analysis**

Small quantities from 4C, 12C, 30C, 200C, 1M, 10M and 50M of ultra-high diluted succussed solutions were subjected to granulometric nano-analysis and their corresponding z-potential was measured. The results are summarized in Figure 4. From Figure 4a is derived, that the size of particles in all liquid samples are in the range of micro  $\mu\text{m}$ ( $\mu\text{m}$ ) to the nanoscale. Similar results are referred also by others, by the use however of different methods of analysis [28,29]. Figure 4a shows z-average size (dnm) distribution (also known as the “cumulants mean”) before and after succussion during preparation process from 4C up to 50M sample. Succussion is a vigorous agitation of the liquid during preparation of the UHDSSP. In this Figure, the role of succussion is clearly illustrated. It appears that the differentiation among substance size's increases after succussion, with the greatest at 1M solution product. From the plot before succussion (black line), it is obvious that there is a small change of the particle's size for solutions 4C up to 10M and after this, there is a slight diminution. The red plot shows an augmentation from 4C to 12C potency and a small drop from 12C to 200C. Then, an exponential augmentation from 200C to 1M potency occurs and after that, a small drop up to 50M is observed. Figure 4b shows Pdi distribution before and after succussion of our liquid samples. After succussion (Red Plot) the polydispersion of the particles of AM presents a small increase from 4C to 12C and then a large decrease until 1M, where an exponential diminution with the maximum at 1M is observed. These fluctuations of polydispersed features correspond to the fluctuations in the diverse potencies as well. Succussion increases or reduces the certainty particles contained that any drop from whichever UH-DSSP will contain particles [30].

**Zeta potential analysis**

Zeta Potential is a method showing the degree of stability of a colloid system. Figure 4c demonstrates the differentiation of zeta potential (mV) before and after succussion, during preparation process from 4C up to 50M solution. The Zetasizer system determines the size by first measuring the Brownian motion of the particles in a sample using DLS and then interpreting a size from this using established theories. DLS and ZP measurements have gained popularity as simple, easy and reproducible tools to ascertain particle size and surface change. In all potencies, the polarity of zeta potential follows its negative course which is lower than the equilibrium states. One of the most popular uses of ZP data is to relate them with colloid stability [31]. Guidelines classifying NP dispersions with ZP values of  $\pm 0-10\text{mV}$  (highly unstable),  $\pm 10-20\text{mV}$  (relatively stable),  $\pm 20-30\text{mV}$  (moderately stable) and  $\pm >30\text{mV}$  as highly stable, respectively are common in drug delivery literature [32]. Thus, according to the latter, some solution products, before succussion seem to form relatively stable colloids, while the more highly unstable being the 4C, 12C, 200 C and 10M solution products. After succussion, zeta potential plotted in all samples between -2,91 up to -11,1 demonstrates that all samples are forming highly unstable colloids except the case of 50M which form a relative stable colloid system. Also, the relation between ZP and particle concentration is complex and usually determined by both surface adsorption and the effect of EDL. When a charged particle is dispersed in a medium, an adsorbed double layer often referred as EDL develops on its surface [33]. It is difficult to provide general guideline about the effect of concentration on ZP. However, it can be stated that over all in dilute conditions like UHDSSP, the surface adsorption phenomenon dominates and hence, ZP increases with increasing concentration. However, at higher concentration range, the thickness of EDL dominates and then by increasing concentration an opposite effect i.e. decreases in ZP with lesser stability of the dispersion is observed [34]. It is obvious that nano-dimensional is the one that predominates in these solutions from which the AM's root is prepared as UHDSSP. Considering the two plots of Figure 4c, it is obvious that the fluctuations of both plots were generally similar except from the final point for 50 M potency. The succussed 50M seemed to lose its stability as a colloid system while the unsuccussed 50M presented a much better stability, according to the above mentioned. It was not surprising, if a closer look at the corresponding particle size is given. The sizes were almost the same up to potency 200C included. After that, for potencies 1M, 10M, 50M there was a dramatic change/increase of size with the maximum attained, almost at 10M until 50 M. Potency 200C may be assumed that was the turning point for succussion also, as every change looks like beginning there, as it did in all previous plots, of Figures 4a and 4b. Therefore, ZP analysis proved the colloidal dispersion of AM particles in all potencies. The existence of a colloid system requires colloidal dimensions

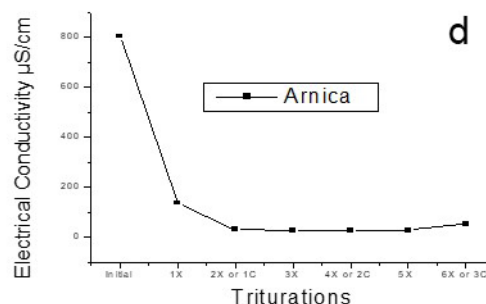
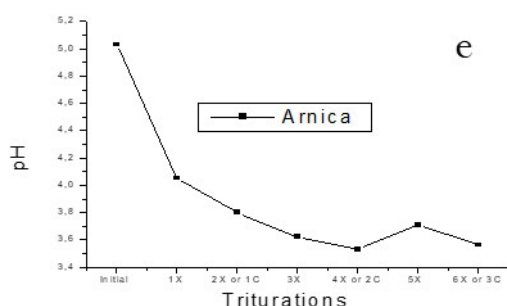


**Figure 4:** (a) Z-Average size (dnm), (b) Pdi, (c) Zeta Potential (mV) distribution before and after succussion of Arnica's Montana samples

i.e. 1nm to 1  $\mu\text{m}$ , as the AM particles are after trituration and succussion.

### Electrical conductivity - pH analysis

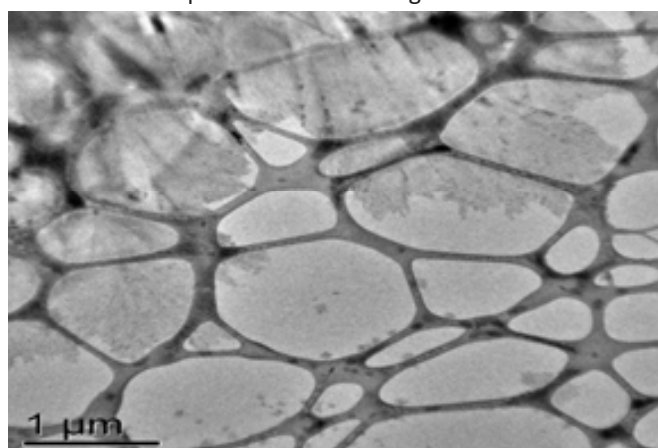
The properties of all solutions have been investigated by measurements of their electrical conductivity and pH values. For each of the prepared samples (trituated and then turned in solutions) three measurements were effectuated and the average value was calculated for each one. The results are shown in Figures 5d,5e. A remarkable change\decrease in electrical conductivity is noticed, from the initial RSM up to the 4X trituration and a slight relative increase in 5X and 6X triturations is observed. A similar behavior is noticed for their corresponding pH values. A gradual reduction of the pH up to 4X, a small rise in 5X and a new small drop in 6X trituration is observed. The observed differences must be attributed partly to the presence of  $\alpha$ -lactose monohydrate in the prepared samples, and partly to the nanosized AM particles. All these changes were not expected, as no other substances (acids or alkali or salts) were added or removed from the samples themselves, i.e. having in mind that for  $\alpha$ -lactose monohydrate the pH value is 4,03 if dissolved in water. The only thing that had occurred during the trituration process of AM was the size of its particles which has been significantly decreased in the micro and nanoscale. The same measurements were made for 12C, 30C, 200C, 1M, 10M and 50M solutions. All the received results were the same as the solvent. Figure 5d revealed a decrease in electrical conductivity quite intense for 1X compared to the one of the initial RSM. This may be attributed to the presence of  $\alpha$ -lactose monohydrate (organic, molecular substance) which is not a substance with electron carriers or of any other kind of ions. The decrease continued to occur more slightly, up to 5X trituration in  $\alpha$ -lactose monohydrate almost with the same values (30-27. -5  $\mu\text{S}/\text{cm}$ ). The surprise was that for trituration 6X, where the active ingredient is much less than the previous triturations, electrical conductivity increased. The observed decrease of pH value for the first trituration, 1X was attributed to the presence of  $\alpha$ -lactose monohydrate. For triturations beyond 1X, the pH value decreased regularly by 0.2 reaching at 6X trituration, a quite acidic value of 3.56, i.e. the pH had decreased about one and a half unit. For triturations 1X to 6X, the pH values were found smaller about 0.5 unit, if compared with the one of the initial RSM. The changes noticed for electrical conductivity and for pH values, were 93,45 % and 29,22 %, respectively, leading to the conclusion that trituration results to a 6X sample (mixture of  $\alpha$ -lactose monohydrate and AM) with completely different/new properties. The observed differentiation of properties may be attributed to the dramatic diminution of particle size (nanoscale), as well as to the presence of new functional groups after trituration.



**Figure 5:** Measurements of Elect. Conductivity (d), pH (e) for initial RSM up to 6X triturations

### TEM analysis

In order to confirm the presence of nanoparticles in the samples, we performed TEM characterization after the succussion of the 50M potency. As it can be seen from the TEM image in Figure 6, there is organic matter, with particle sizes ranging from a few nanometers up to 8 micrometers in the form of agglomerates. AM's particles are easily distinguished having a darker color on the surface of the grid. The size range of the particles seen in TEM image, agrees well with the results obtained by DLS method after sample's succussion in Figure 4a.



**Figure 6:** Arnica's Montana 50M TEM image in 1  $\mu\text{m}$  scale.

### Conclusion

During this study, a number of instrumental methods of analysis have been used in order to investigate the changes in the physicochemical properties of Arnica as an ultra-high diluted succussed solution solid origin product from the beginning up to 50M solution and the obtained results are presented. These changes are considered to be related to the gradual change in the size of the material during trituration. The experimental findings confirm the existence of the Arnica's ingredients in all ultra-high diluted succussed solution products. It was also found that beyond the reduction of grains size to a nano dimensional scale up to 6X trituated step, there is an appearance of functional groups in organic compounds in our mixture that did not exist in the initial sample. It seems that in the case of Arnica the trituration in  $\alpha$ -lactose monohydrate works mechanochemically. There appears to be a change in the basic physiochemical properties such as the change in Electrical Conductivity and pH. It is obvious that nano-dimension is the one that predominates in these solutions. Thus, the succussion of the solutions leads to the formation of aggregates when the particles are in the nanoscale. On the contrary when aggregates are already present, succussion turns them into smaller particles. Also, zeta po-



tential measurements confirm the colloidal or not nature and stability or not, of the solutions. The conditions and the quality of the preparation process have a key role for qualitative ultra-high diluted succussed solution products. Since this type of research is being conducted for the first time, further investigation into other similar products is recommended. Eventually, the trituration in  $\alpha$ -lactose, the dilution and the succussion, are simple methods to prepare nanoparticles.

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