

Article

An Exploratory Study of Electrophotonic Analysis in Size-6 Pilules Impregnated with Stepwise Dilutions of *Cuprum metallicum* and *Gelsemium sempervirens*

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Abstract

Background: The characterisations of highly diluted preparations impregnated onto solid carriers remains analytically challenging, particularly when residual material is present at very low levels. Electrophotonic analysis (EPA), based on corona-discharge imaging and quantitative image analysis, has recently been explored as a possible complementary approach, but its applicability to impregnated pilules requires further investigation. **Purpose:** This exploratory study examined whether EPA-derived image parameters could reveal measurable differences among size-6 pilules impregnated with stepwise diluted and dynamised preparations of *Cuprum metallicum* and *Gelsemium sempervirens* and whether such differences might vary according to source material and manufacturing protocol. **Methods:** Size-6 pilules were impregnated with Hahnemannian and Korsakovian preparations of *Cuprum metallicum* and *Gelsemium sempervirens*. Control samples included non-impregnated pilules, pilules impregnated with pure solvent, and pilules impregnated with simply diluted, non-dynamised preparations. Images were acquired in randomised and blinded conditions using a prototype EPA device. Image intensity, contrast, entropy, and fast Fourier transform (FFT)-derived period/spatial-frequency parameters were analysed. **Results:** The EPA images showed patterns that may indicate differences between impregnated pilules and controls, between the two source materials, and between manufacturing protocols under the experimental conditions used here. FFT-based analysis suggested that some spatial-period/spatial-frequency indices may contribute to describing these preliminary differences. Among the parameters examined, the smallest diameter of the selected high-intensity FFT-image regions remained statistically significant after correction for multiple comparisons, whereas other parameters should be interpreted cautiously. Observations of aged samples also suggested possible differences from reference preparations, but these findings remain preliminary and require confirmation. **Conclusions:** These results suggest that EPA combined with FFT-based image analysis may provide exploratory information on EPA-derived image patterns in potentised preparations impregnated onto solid carriers. However, the present data are not sufficient to establish EPA as a reliable discriminatory analytical tool. Further measurements, larger sample sets, independent replication, improved control of environmental variables, and comparison with complementary analytical methods are required before firm conclusions can be drawn about its analytical value.



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Keywords: electrophotonic analysis; corona discharge; discriminatory potential; photon electrochemistry; impregnated solid matter; Cuprum metallicum; Gelsemium sempervirens

1. Introduction

Conventional analytical approaches for detecting trace amounts of active constituents remain highly valuable, but they also have significant limitations when analyte concentrations become extremely low. Electrochemical methods can offer good sensitivity for selected analytes; however, their performance often depends strongly on electrode properties, matrix composition, interfacial phenomena, and signal stability. In complex or weakly reactive systems, these factors may reduce selectivity and reproducibility, particularly when the analyte is present near the limit of detection [1–3].

Similarly, spectroscopic methods remain central to modern analytical chemistry; however, their application to extremely low analyte concentrations often requires extensive sample preparation, preconcentration, or highly specialised instrumentation. In practice, matrix effects, background noise, spectral overlap, contamination, and detection-limit constraints can complicate the reliable identification of ultratrace constituents, particularly in heterogeneous or weakly emissive materials. Even highly sensitive techniques generally require strict experimental control to distinguish genuine analytical signals from instrumental or environmental artefacts [2,4–6].

Within this context, electrophotonic analysis has emerged as an experimental and still-developing approach based on corona-discharge imaging and quantitative image analysis. Earlier applications of electrophotonic imaging were reported mainly in biological and environmental contexts, including studies involving humans, plants, water, and other living or natural systems, particularly within the GDV/EPI framework developed by Konstantin Korotkov [7,8].

At its current stage, this approach should not be viewed as a replacement for established analytical methods, but rather as a complementary technique that may provide additional discriminatory information in hydrated materials or in solid supports impregnated with low-dose preparations. Recent work [9] has focused on the reproducibility of the method, the characterisation of emission patterns, and the exploration of possible analytical applications in liquids and solid supports. Its present use therefore remains essentially exploratory, and further technical refinement, validation, and inter-method comparison are still needed to clarify its analytical scope and limitations.

The purpose of this study was to examine whether electrophotonic analysis (EPA) could reveal measurable differences between different types of source materials diluted in hydro-alcoholic solutions and subsequently impregnated onto size-6 pilules. EPA is based on corona discharge imaging and records changes in emission patterns that may be influenced by the physicochemical state of the sample and its interaction with the surrounding atmosphere. A large set of image-analysis techniques was applied to extract information encoded in EPA images. The earlier EPA study [9] suggested that corona discharge patterns may reflect changes in both liquid water and interfacial water, two essential components influencing electro-photon emission [10].

2. Experimental Design

In an electrophotonic experiment, a piece of condensed matter, either liquid or solid, is placed on a transparent indium tin oxide (ITO) anode, with a cathode needle in contact with its upper surface (see Figure 1). This configuration allows for a very low-amperage, high-voltage electrical signal, at a specific frequency and waveform, to be applied to the

anode. When the electrical potential reaches a critical threshold, millions of electrons are pulled out of their orbitals in the anode, collide with surrounding atoms, and displace additional electrons. This electronic activity ionises the surrounding air, creating negative and positive electrical pathways along which ions can travel. Lightning-like streamers then appear in the visible and ultraviolet light spectrum (corona discharge) and can be recorded with a CCD camera.

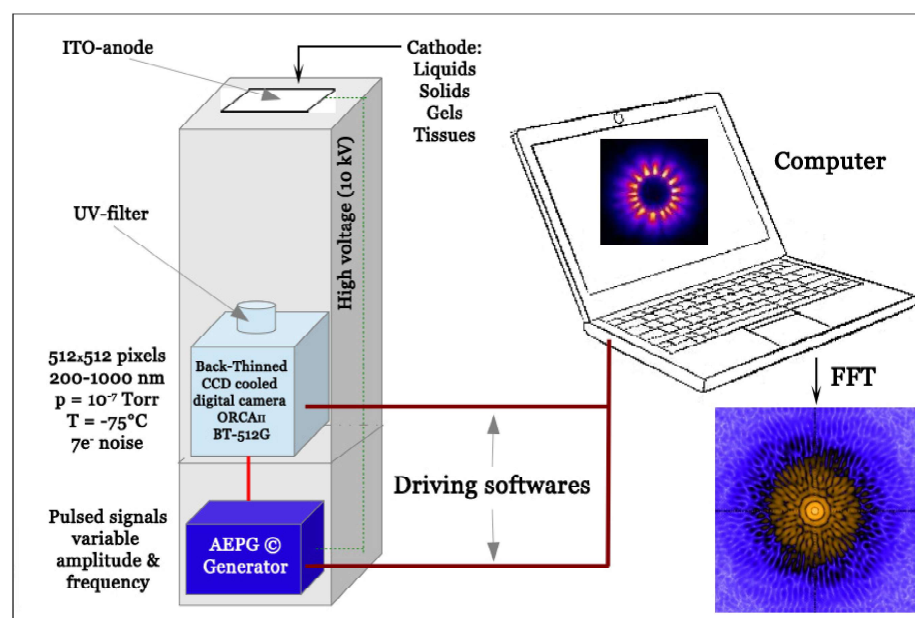


Figure 1. The principles of the corona effect and the experimental device for recording electrophotonic images.

Our measuring device is based on an advanced electrophotonic generator (AEPC©) that can produce a stable and reproducible electromagnetic field in terms of both voltage and frequency. Coupled with other components controlled by highly reliable electronics, this generator produces an electromagnetic field on the electrode plate with its strong pulse voltage. This field alternates between positive and negative charges at a predefined frequency. This field successively mobilises electric charges on the surface and within the object to be analysed, causing ionisation of the gaseous environment around the object (plasma gas). This ionisation creates an electronic avalanche, splitting the gas molecules and releasing UV photons, which are recorded by the Hamamatsu camera. These phenomena do not appear simultaneously, but one after the other, depending on the pulse generator. Image acquisition provides an idea of the statistical distribution of light emission during exposure time. Numerous experiments have shown that charges are distributed primarily in two ways:

The generator produces positive pulses that lead to the formation of filamentary structures called “streamers”.

Negative pulses create rounded and globular forms known as “coronae”.

These acquisitions allow the increasing richness of the image to be assessed as the complexity of the analysed object increases.

Attached to this generator is an EFUSE© (EFUSE GmbH, Solingen, Germany), a transparent and conductive electrode plate with the highest possible homogeneity across its entire surface, which allows for precise control over the distribution of electrical charges. To record the photonic emission, we used a Hamamatsu ORCA IIBT 512G2 HD (Hamamatsu Photonics K.K., Hamamatsu City, Japan) camera with specific timing and adjustable exposure time, coupled to an optical system equipped with a UV filter.

It is worth noting that many physical environmental factors must be taken into account when conducting electrophotonic experiments. These include ambient atmosphere (gas), moisture (a crucial factor for ionisation) and dust (highly sensitive to electric fields).

3. Results

EPA measurements suggested potential differences between impregnated pilules, controls, and between the two source materials. Non-impregnated pilules and solvent-impregnated pilules showed different baseline values. Potentised samples exhibited higher variability, as expected from complex material structures; however, these observations remain exploratory and should not be interpreted as validation of EPA as a definitive discriminatory method.

For *Cuprum metallicum*, both Hahnemannian and Korsakovian methods produced EPA images with identifiable trends across dilutions. Ageing did not appear to reduce photonic energy or contrast; in some cases, entropy increased, which may suggest long-term changes in EPA-derived image features.

For *Gelsemium*, the behaviour differed from *Cuprum metallicum*, with different patterns of energy, contrast, and entropy. Dynamised samples showed lower values than simply diluted controls, which may indicate that the preparation process is associated with changes in EPA-derived image parameters.

Fast Fourier transform (FFT) analysis showed period/spatial-frequency distributions across dilutions within each production line. Within this dataset, these distributions differed between *Cuprum* and *Gelsemium*, but further independent validation is required before such differences can be used for prospective classification of unknown samples.

Overall, these preliminary findings suggest that EPA may be useful for detecting differences associated with very low amounts of material, although further validation is required.

3.1. Analysis of Images and FFT of These Images

For this initial study, we acquired 210 EPA images of size-6 pilules, including complete production lines from 4 to 30 cH for *Cuprum metallicum* and from 4 to 30 cH for *Gelsemium sempervirens*, together with control groups consisting of non-impregnated pilules, pilules impregnated with pure solvent, and pilules impregnated with simply diluted *Cuprum* or *Gelsemium* preparations (see Figure 2 for the controls and the 30 cH samples as examples). Three measurements were performed on randomly selected pilules from numbered containers, each containing 80 pilules: one container of non-impregnated pilules (dry blank control), one container of pilules impregnated with pure solvent (solvent control), two containers of aged impregnated pilules, one prepared according to the Hahnemannian process (cH) and one according to the Korsakovian process (cK), six *Gelsemium* cK samples, six *Cuprum* cK samples, 27 *Gelsemium* cH samples, and 27 *Cuprum* cH samples.

All images were acquired indoors in an air-conditioned environment (see Table 1), within a single day and in randomised order, thereby minimising the influence of external factors.

Only ultraviolet emissions were captured by the system. Image analysis was performed using FIJI (<https://fiji.sc/>, accessed on 25 August 2026) [11]. The original EPA images acquired with the Hamamatsu camera were 16-bit images and were used for the calculation of image-derived parameters such as energy, contrast and entropy. FIJI first generated the FFT from the original EPA photograph provided by the EPA device. The source photographs were directly comparable from one image to another since each pilule was consistently placed at the centre of the ITO anode (512×512 pixels; 50×50 mm), and all images had the same dimensions, corresponding to the full surface of the ITO anode.

The Hamamatsu camera settings were kept constant throughout the study to provide sharp, high-quality images without subsequent correction.

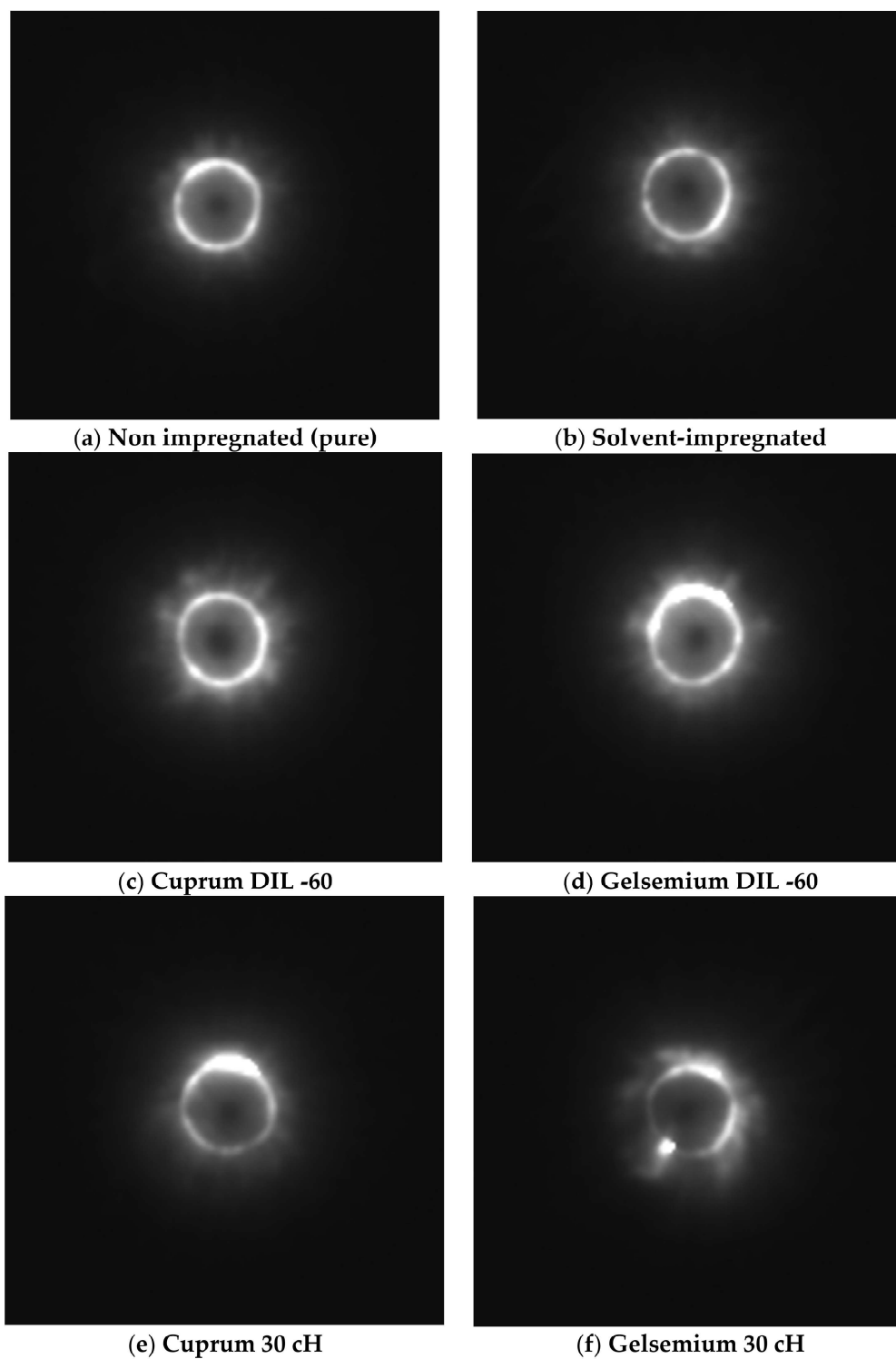


Figure 2. *Cont.*

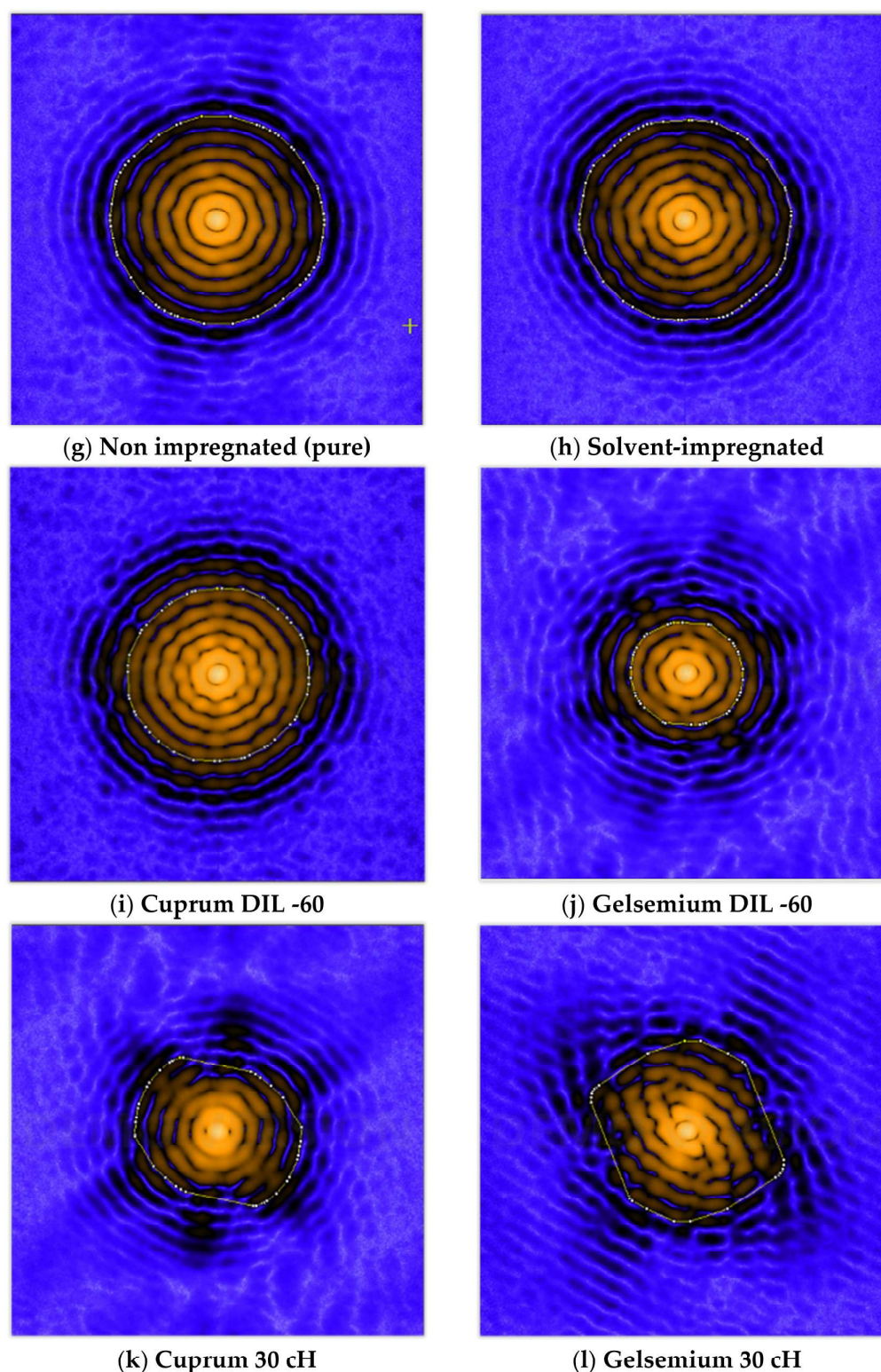


Figure 2. Representative source images and corresponding FFT-derived electrophotonic profiles of size-6 pilules, either non-impregnated, impregnated with pure solvent, or impregnated with a dynamised or diluted solution. The dashed outline in the FFT images indicates the region containing the highest-intensity spatial-frequency components.

For FFT-based region selection, the FFT images were visualised in FIJI using the standard “Fire” look-up table and handled as 8-bit display images for segmentation purposes. In this 8-bit FFT representation, pixel grey-level values range from 0 to $2^8 - 1 = 255$. To improve reproducibility, the central FFT region was isolated using the same LUT, with the

segmentation boundary set at the transition to the orange/yellow region. This visual boundary corresponds to a high-intensity 8-bit grey-level threshold of ≥ 120 on a 0–255 scale. This criterion filters out low-intensity diffuse frequencies and peripheral background noise, rendered in black, blue and purple, and retains the core high-intensity central cluster. The total area of this segmented FFT region was then automatically computed in px^2 to derive an equivalent FFT-feature dimension. When the shape was not circular and did not form complete rings, only clearly defined orange regions were selected.

Table 1. The recorded temperatures and relative humidity levels in the laboratory are shown during acquisition of the electrophotonic images. The voltage was 11 kV at a frequency of 120 Hz for size-6 pilules.

Date	15 April 2016
T/°C	21–24
R.H. %	42–46

Table 2 summarises the mean area of the most intensely illuminated regions selected in the FFT images. The second numerical column gives the square-root equivalent dimension of each selected FFT region, calculated as $\sqrt{\text{area}}$ and expressed in pixels. This value is an equivalent linear dimension of the selected FFT feature and should not be interpreted as the radial distance from the centre of the FFT image to a discrete frequency peak. Accordingly, the corresponding spatial periods and spatial frequencies are presented as descriptive FFT-derived indices rather than as direct centre-to-peak frequency measurements. The selected FFT regions correspond to the most intense low-spatial-frequency components observed in the transformed images. These values do not allow for a direct correlation with UV wavelengths. However, the recorded emission was restricted to the UV spectral range because the camera filter allowed only this range to be visualised.

Using FFT-derived images, we found that several parameters were potentially discriminative within this dataset (see Table 3 for an example). These parameters were calculated from the FFT-image region corresponding to the most intense signal and the longest spatial period, i.e., the lowest spatial frequencies. Visual inspection of the source images suggested qualitative differences; however, FFT transformation enabled quantitative statistical analysis that was consistent with these observations (see Supplementary MANOVA Data). A specific entropy-related image structure was observed for size-6 pilules impregnated with potentised solutions, but this finding should be interpreted as exploratory and hypothesis-generating.

For each of the 27 potentisation stages (from 4 CH to 30 CH), a single sample was analysed using image analysis. From the distribution of the diameters of selected features in the FFT image, three metrics were extracted to characterise the distribution profile: Max (maximum diameter, MinFeret), Min (minimum diameter, MinFeret), and Middle (median diameter value of the analysed structures). These three values do not represent independent biological replicates, but rather a multivariate description of FFT-derived image features for a single preparation at a given stage.

The MANOVA statistical analysis of the full manufacturing lines data (cH) revealed significant differences: there is a significant difference ($p = 0.002$) between full manufacturing lines of Gelsemium and Cuprum for the diameter of the selected area (most illuminated FFT area). There is a significant difference ($p = 0.001$) between Gelsemium and Cuprum for the smallest diameter of these selected areas, which can be converted into period/spatial frequencies, but no significant difference for observed angles and other parameters.

Table 2. Mean area of the most intensely illuminated FFT regions, square-root equivalent FFT-feature dimensions, and derived comparative spatial-period/spatial-frequency indices.

Sample of Impregnated Size 6 Pilules	px ²	Equivalent FFT-Feature Dimension $\sqrt{\text{Area}}$ (px)	Mean FFT-Derived Spatial-Period Index (μm)	Spatial-Frequency Index (Cycles/mm)
Non-impregnated	186,771	432.17	116 μm	8.62 cycles/mm
Impregnated with solvent	117,280	342.46	146 μm	6.85 cycles/mm
Cuprum CH (mean)	45,082	212.33	235 μm	4.26 cycles/mm
Gelsemium CH (mean)	61,405	247.80	202 μm	4.95 cycles/mm
Cuprum simply diluted	11,6686	341.59	146 μm	6.85 cycles/mm
Gelsemium simply diluted	31,860	178.49	280 μm	3.57 cycles/mm

Table 3. Smallest diameter of the selected high-intensity FFT-image regions, expressed in μm, for three measurements of Gelsemium and Cuprum across 4 CH–30 CH. For each potentisation stage, the lowest value was classified as Min, the highest as Max, and the remaining value as Middle.

Potentisations	4 CH	5 CH	6 CH	7 CH	8 CH	9 CH	10 CH	11 CH	12 CH	13 CH	14 CH	15 CH	16 CH	17 CH	18 CH	19 CH	20 CH	21 CH	22 CH	23 CH	24 CH	25 CH	26 CH	27 CH	28 CH	29 CH	30 CH
Min Gels	248.259	283.995	224.635	294.329	234.638	268.036	246.651	221.550	241.913	271.216	194.151	249.251	238.847	255.408	275.234	221.904	253.391	236.532	239.485	229.703	228.520	208.923	269.126	277.357	238.868	254.306	244.138
Middle	296.520	284.724	227.455	294.360	253.207	286.437	247.045	223.633	241.913	272.000	203.198	246.538	236.029	255.691	285.084	229.191	271.036	254.248	245.301	247.236	228.694	220.028	277.744	277.357	240.630	254.659	245.418
Max Gels	268.349	290.999	241.387	295.500	261.041	299.039	247.045	233.650	242.374	283.848	203.988	249.682	242.068	255.794	285.905	230.734	271.299	254.730	246.782	247.488	229.703	224.104	278.970	277.673	241.224	255.088	246.122
Min Cupr	274.408	234.861	275.877	264.686	282.423	285.549	282.551	243.613	278.148	261.515	274.197	253.482	291.234	274.666	234.134	256.874	292.081	275.375	302.053	255.218	267.709	269.945	257.474	280.110	280.858	270.831	281.516
Middle	282.033	235.659	276.047	278.607	283.398	289.850	282.929	257.223	278.271	261.717	274.427	253.482	291.238	275.081	234.609	258.477	292.458	277.023	302.149	266.169	268.140	270.228	258.861	280.110	294.422	271.052	281.649
Max Cupr	291.709	235.847	276.570	281.368	283.688	290.614	283.550	257.583	286.833	277.587	274.721	254.102	291.238	280.033	235.427	259.905	292.905	279.573	302.149	276.438	268.561	274.325	259.399	281.485	295.120	271.334	287.000

Because 30 image-derived parameters were examined, correction for multiple comparisons was required. Applying the Bonferroni correction to a p -value of 0.001 across 30 tests gives either an adjusted p -value of 0.03 (0.001×30) or an adjusted significance threshold of 0.00167 ($0.05/30$). In both cases, the result remains statistically significant since $0.03 < 0.05$ and $0.001 < 0.00167$. Thus, the smallest diameter remains statistically significant and warrants further investigation. By contrast, for the diameter parameter ($p = 0.002$), the Bonferroni-adjusted value is 0.06, and $0.002 > 0.00167$; therefore, this result is no longer statistically significant after correction.

We added (Table 4) a paired multivariate analysis test (Hotelling's T^2 MANOVA by blocks) to the three dimensions simultaneously (Max, Middle_3, Min). Multivariate analysis of variance using a blocked design reveals the following results regarding statistical significance for each parameter.

Table 4. Results of the paired multivariate analysis of the smallest diameters of the selected high-intensity FFT-image regions across the three measurements.

Response Variable	GELS Mean ($\pm$ SD)	CUPR Mean ($\pm$ SD)	Statistic Paired (t)	p -Value (Significance)
Max μm	258.1 μm (± 45.9)	219.8 μm (± 28.1)	$t = 3.74$	$p = 0.0009$ (highly significant)
Middle_3 μm	247.0 μm (± 45.7)	215.1 μm (± 27.9)	$t = 3.24$	$p = 0.0033$ (highly significant)
Min μm	239.0 μm (± 45.8)	210.0 μm (± 28.0)	$t = 2.93$	$p = 0.0070$ (very significant)

Global MANOVA test (Wilks' Lambda): $F(3, 24) = 5.61$; $p = 0.0047$.

Brief conclusion: In this dataset, the difference between GELS and CUPR was statistically significant overall ($p < 0.01$) throughout the step-by-step preparation. The GELS preparation showed higher average FFT-derived feature dimensions across the 27 potentiation stages, whereas CUPR showed lower values. The GELS curve also displayed a wider range of fluctuation from one stage to the next, whilst CUPR exhibited a narrower and smoother profile around its mean. These differences should be interpreted as exploratory EPA-derived patterns rather than as proof that independent unknown preparations can be classified reliably.

This analysis was complemented by trend equations using a fixed model. The interaction term suggested different trajectories for the two products across the stepwise dilution series (interaction $p = 0.004$). Within the present dataset, the two products therefore appeared to show different EPA-derived evolution profiles during the potentiation process. GELS tended to increase gradually, whereas CUPR showed a tendency towards reduced FFT-derived feature dimensions as dilution increased. These trends require confirmation in independent production lines before they can be considered robust analytical markers.

In Table 2, the mean surface area of the most illuminated FFT region for the control non-impregnated size-6 pilules was 186,771 px^2 (" px^2 " denotes pixels squared, or the number of pixels in a selected area); for the solvent-impregnated size-6 pilules, it was 117,280 px^2 . For Gelsemium, the mean was 61,405 px^2 , and for Cuprum, it was 45,082 px^2 . Within this exploratory dataset, these values suggest differences between pilules impregnated with potentised preparations and those impregnated with pure solvent in selected FFT-derived image features. These observations should not be interpreted as proof that potentised preparations are generally distinguishable from solvent controls. For the simply diluted preparations, the area was 31,860 px^2 for Gelsemium and 116,686 px^2 for Cuprum. Simply diluted Cuprum showed values close to the solvent-impregnated reference, whereas the

most illuminated FFT area for simply diluted Gelsemium was smaller than the dynamised mean (see Table 2).

In Table 2, the average distribution of the shorter diameter of the selected FFT-image region (see Figure 3, including the cumulative data because in such a manufacturing process each dynamisation depends on the content of the previous one), which corresponds to an FFT-derived spatial-period index, was 202 μm for Gelsemium and 235 μm for Cuprum metallicum. MANOVA for these period-index values gave $F(3, 24) = 5.61$; $p = 0.0047$. This indicates a statistically significant difference between the group means within this dataset ($p < 0.005$). The FFT-derived period-index values were lower for Cuprum, corresponding to higher spatial-frequency indices, but this should be considered a preliminary image-analysis observation rather than a validated discriminatory criterion.

It should be noted that, for non-impregnated size-6 pilules, the FFT-derived spatial-period index was 116 μm , whereas for dry, neutral size-6 pilules it was 146 μm . The impregnation of potentised solutions of Cuprum or Gelsemium onto size-6 pilules appeared to modify the FFT-derived spatial-period/spatial-frequency indices. We found 280 μm for Gelsemium simply diluted and 146 μm for Cuprum simply diluted, near the value of neutral size-6 pilules. These findings are consistent with the hypothesis that potentised preparations may generate different EPA-derived image features from simply diluted preparations, but this interpretation remains preliminary and requires independent validation.

In the article validating the EPA method [9], Professor Marc Henry proposed examining additional image-derived parameters. That study compared a broad range of image parameters against the principal control condition, which in the present work consisted of size-6 pilules impregnated with pure solvent. When a given value falls outside the control standard-deviation range, this indicates that the corresponding parameter differs from the control. This approach can be useful for the exploratory analysis of small datasets, such as the K-potentiation data in the present study, although the results should be interpreted primarily as indicative and hypothesis-generating. Larger studies will be needed to confirm these preliminary observations.

More generally, blinded acquisition and blinded image analysis ensured that sample identity was concealed during measurement and primary processing, thereby reducing observer-related bias. However, these procedures do not establish EPA as a validated discriminatory or classification method for independent unknown samples. The present study did not include an external validation set, predefined classification rules, or prospective testing of unknown preparations. The observed differences in EPA- and FFT-derived parameters should therefore be interpreted as preliminary and hypothesis-generating until confirmed using larger sample sets, independent production lines and predefined decision criteria.

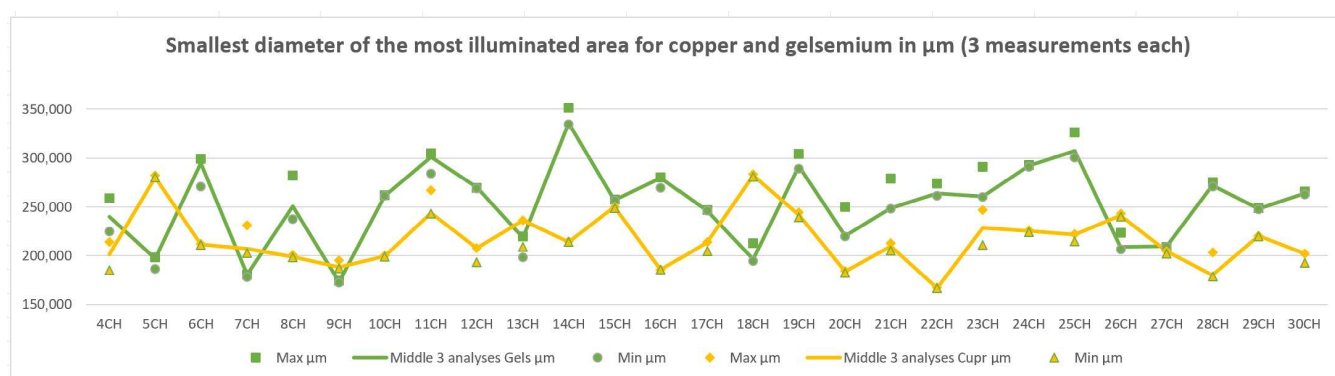


Figure 3. Cont.

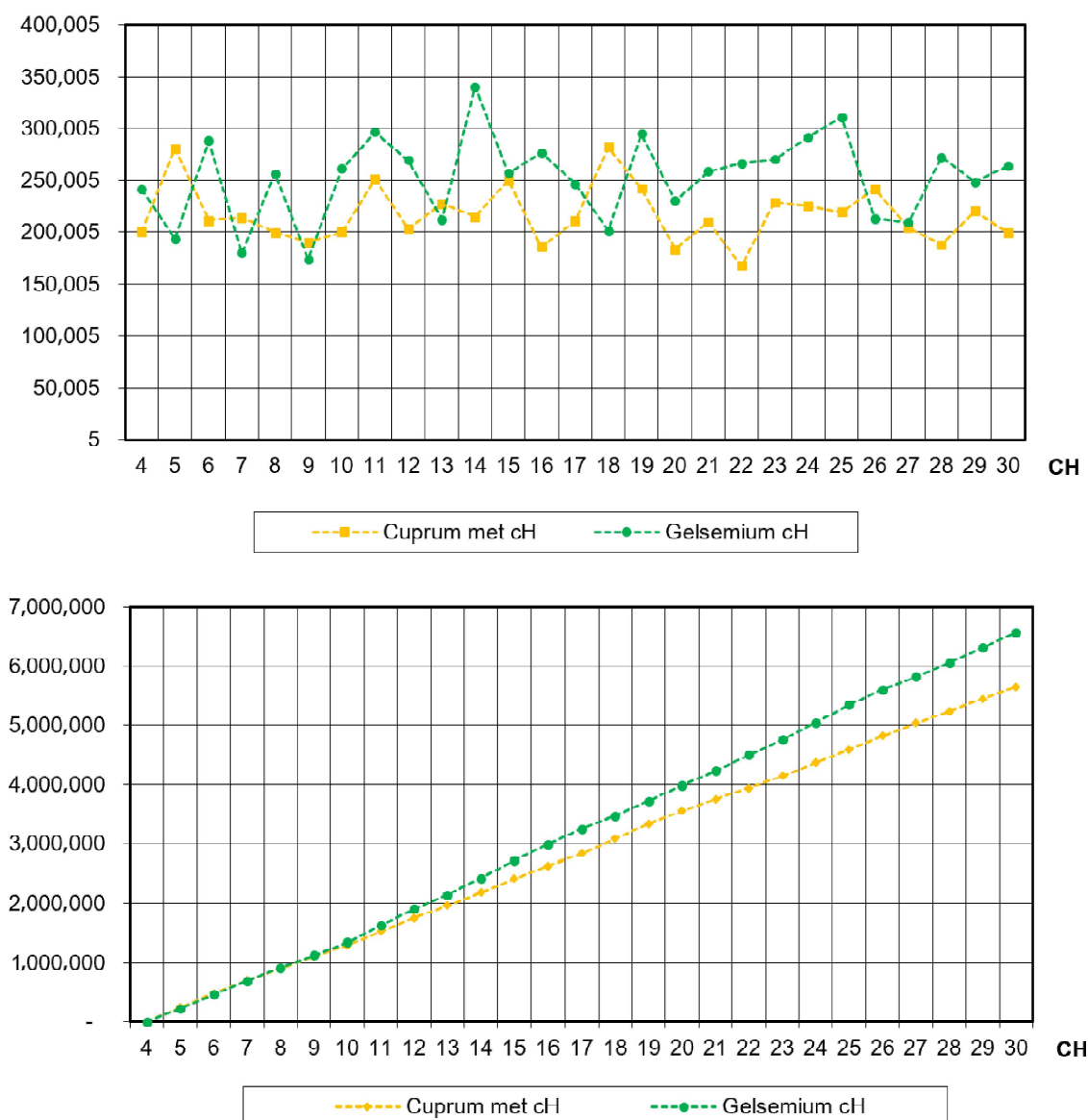


Figure 3. Minimum diameters of the most intensely illuminated regions in the FFT images, measured in triplicate and expressed in μm , for Gelsemium and Cuprum across the full series of impregnated size-6 pilules (4 CH–30 CH). The first panel shows the individual measurements, the second panel presents the corresponding mean values, and the third panel displays the same data as cumulative Lagrangian values.

3.2. Controls

Figure 4 shows the six acquisitions recorded as references for analysing size-6 pilule samples. For the neutral non-impregnated size-6 pilules, after averaging, we obtained $E = 4763$, $\Delta = 2220$ and $S = 1.178$, whereas for the size-6 pilules impregnated with pure solvent, used as the reference condition, we found $E = 4199$, $\Delta = 1885$ and $S = 1.215$. In this exploratory dataset, solvent-impregnated pilules showed higher variability than non-impregnated pilules. Energy and contrast values differed at the descriptive one-standard-deviation level, whereas entropy values did not clearly differ from the reference. $nInt$ denotes the number of grey-level intensity classes detected within the analysed region of the EPA image after image processing. For 8-bit images, pixel grey-level values range from 0 to 255. In this context, $nInt$ provides a descriptive measure of the effective

intensity range or number of occupied intensity levels within the selected region, rather than an independent physical quantity.

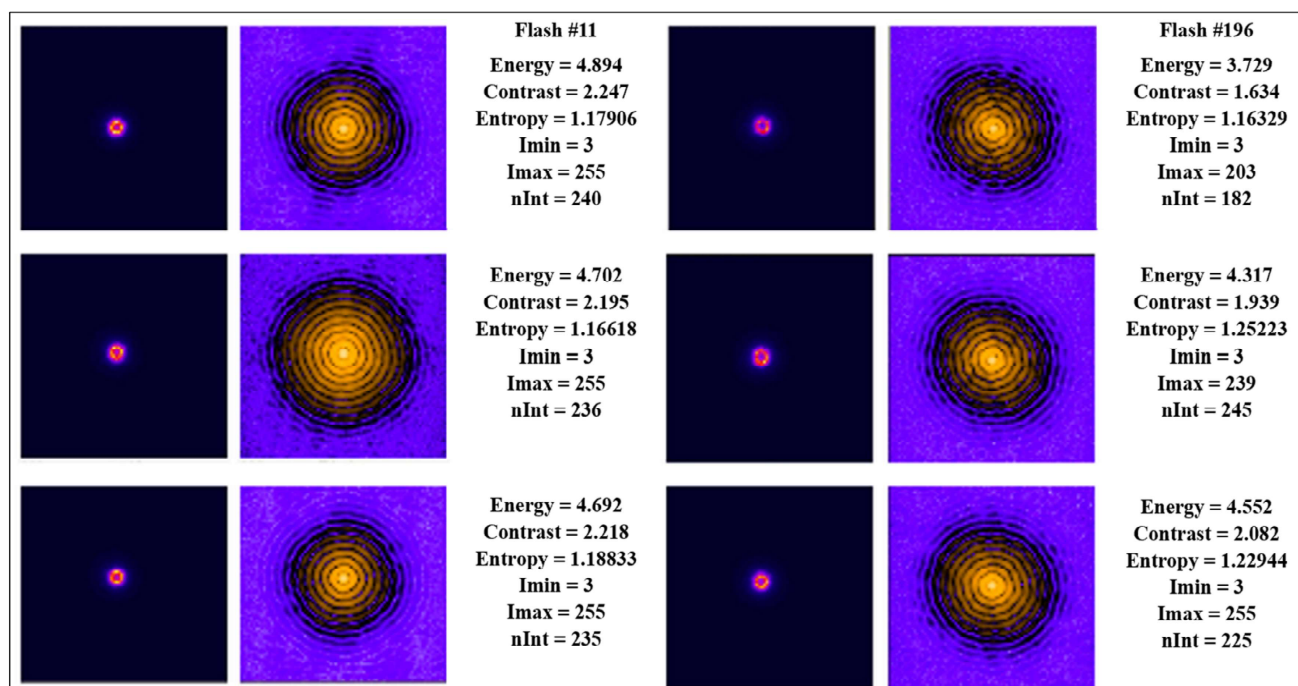


Figure 4. Electrophotonic images with their fast Fourier transform for neutral non-impregnated size-6 pilules (**left**) and size-6 pilules impregnated with pure solvent (**right**). See text for the definition of energy, contrast and entropy. The symbol ‘#’ denotes the sample identification number assigned during blinded coding.

3.3. *Cuprum Metallicum* Size-6 Pilules (Hahnemann Method)

Figure 5 shows representative electrophotonic images recorded on size-6 pilules impregnated with a dynamised *Cuprum metallicum* solution at the 5 CH level. The upper image refers to the preparation made in 2016 for this study, whereas the lower image corresponds to a preparation made 30 years earlier by the same pharmacy. Within these illustrative observations, ageing did not appear to reduce the EPA-derived photonic energy or contrast. The aged sample also showed higher emission intensity and entropy than the fresh preparation. These observations may indicate differences in EPA-derived image texture or signal heterogeneity between the two preparations, but they should not be interpreted as evidence of long-term stability or increased information content. The FFT image suggested a shorter apparent period, corresponding to higher spatial-frequency components in the analysed image features. More measurements are needed in the future.

Figure 6 shows the total energy variations for size-6 pilules impregnated with dynamised *Cuprum metallicum* solutions. The average energy value differed from the pure-solvent reference at the descriptive one-standard-deviation level, although the standard deviation was relatively large. At the individual-sample level, most size-6 pilules displayed energy values outside the reference mean $\pm$ one standard deviation range, except for the 6 CH, 14 CH, 17 CH and 20 CH samples, which remained within this reference range. The distribution showed a small negative skewness relative to a normal distribution, indicating slightly higher energy values at higher dilutions within this dataset. The negative kurtosis relative to a normal distribution indicates that the tails of the distribution were less represented than the central range. These observations remain descriptive and should not be taken as validated discriminatory criteria.

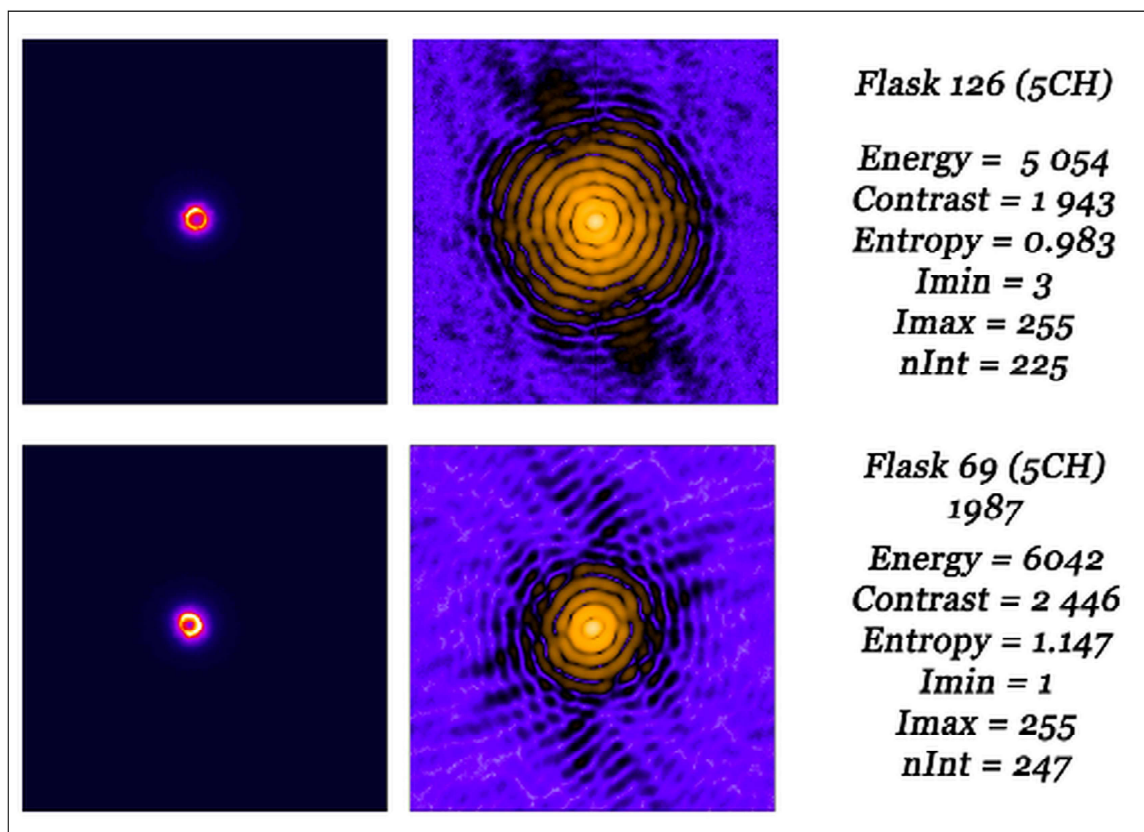


Figure 5. Electrophotonic images with their fast Fourier transform for two potentised preparations of Cuprum metallicum 5 CH made in 2016 (top) and 1987 (bottom).

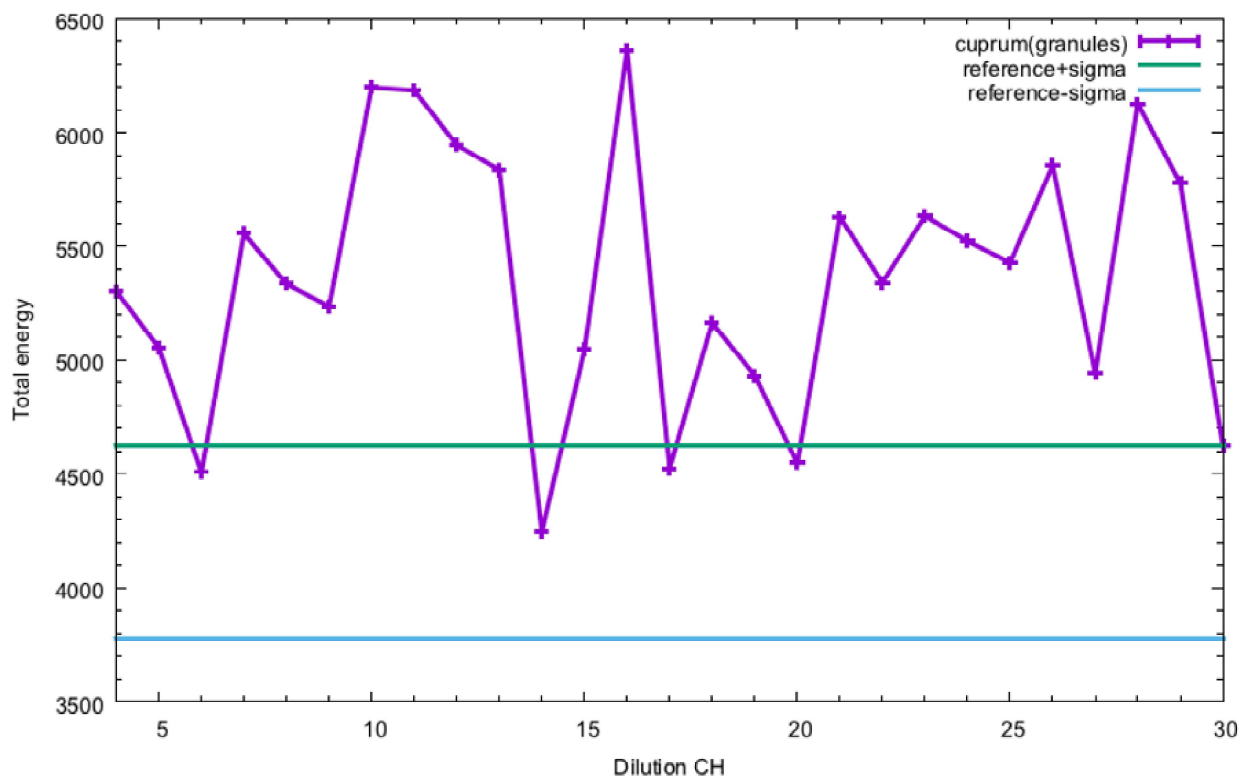


Figure 6. Variation in the total energy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised Cuprum metallicum solutions. The average energy value is $E = 5365$ (577), with a skewness of -0.14 and a kurtosis of -1.01 .

Figure 7 shows contrast variations for the same series of images. The average contrast value did not clearly differ from the reference at the descriptive one-standard-deviation level. However, at the individual-sample level, several size-6 pilules displayed contrast values outside the reference mean $\pm$ one standard deviation range, while 10 of the 27 investigated samples remained within the reference range. The distribution again showed a negative skewness relative to a normal distribution, indicating slightly higher contrast values at higher dilutions within this dataset. Negative kurtosis was also observed, indicating that the tails of the distribution were less represented than the central range, as observed for total energy. These observations provide descriptive EPA-derived patterns and require confirmation in independent datasets.

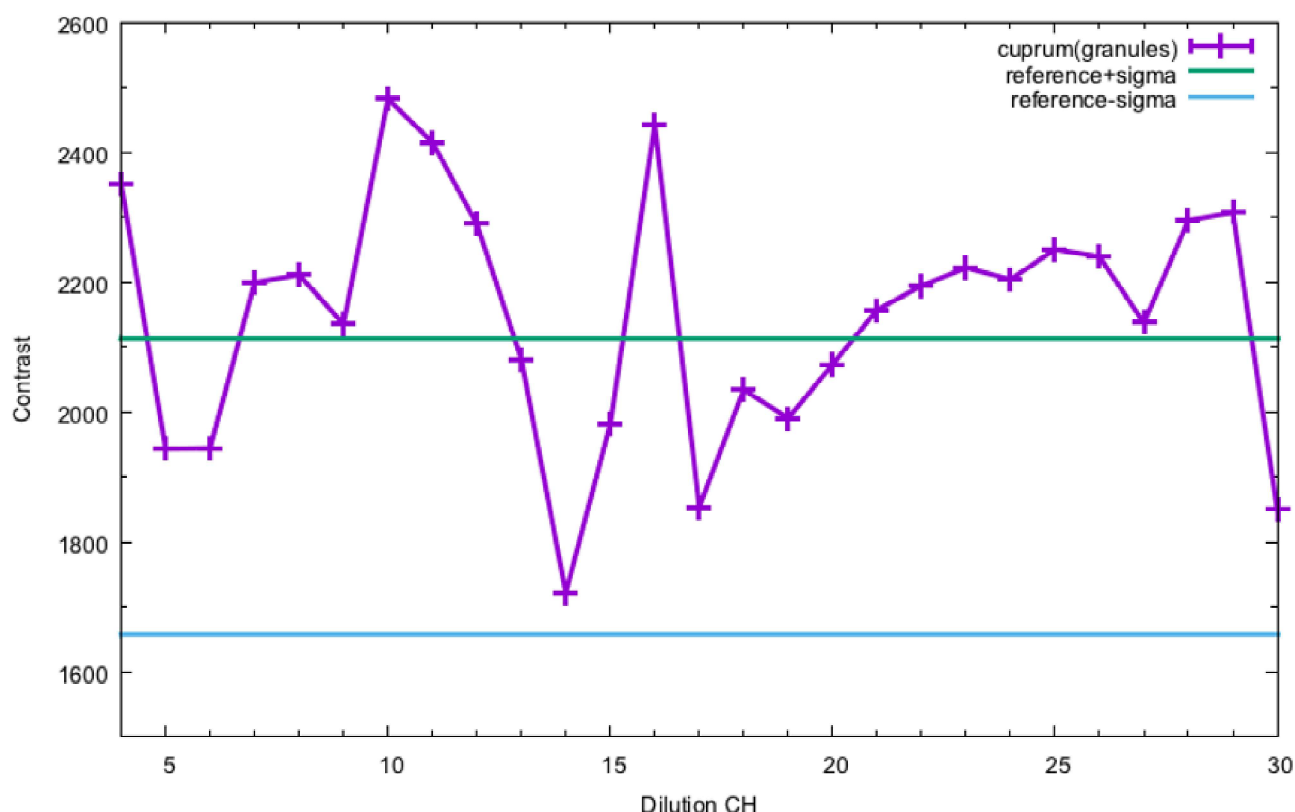


Figure 7. Variation in the total contrast for electrophotonic images recorded on size-6 pilules impregnated with various dynamised Cuprum metallicum solutions. The average contrast is $\Delta = 2148$ (189), with a skewness of -0.30 and a kurtosis of -0.63 .

Figure 8 shows the entropy variations for the same series of images. The average entropy value did not clearly differ from the reference at the descriptive one-standard-deviation level. At the individual-sample level, entropy values were frequently outside the reference mean $\pm$ one standard deviation range, with five samples above and 22 samples below this range. The distribution showed positive skewness relative to a normal distribution, indicating higher entropy values at lower dilutions within this dataset. Negative kurtosis was also observed, indicating that the tails of the distribution were less represented than the central range, as observed for total energy and contrast. These entropy-related patterns should be considered exploratory image-analysis observations rather than validated markers.

Figure 9 compares the electrophotonic images recorded on size-6 pilules impregnated with two simply diluted *Cuprum metallicum* solutions, without dynamisation, with those recorded on size-6 pilules impregnated with corresponding dynamised solutions. In this dataset, the dynamised samples showed lower energy, contrast and entropy values than the

simply diluted samples. These differences should be interpreted descriptively and require confirmation before they can be considered robust effects of the dynamisation process.

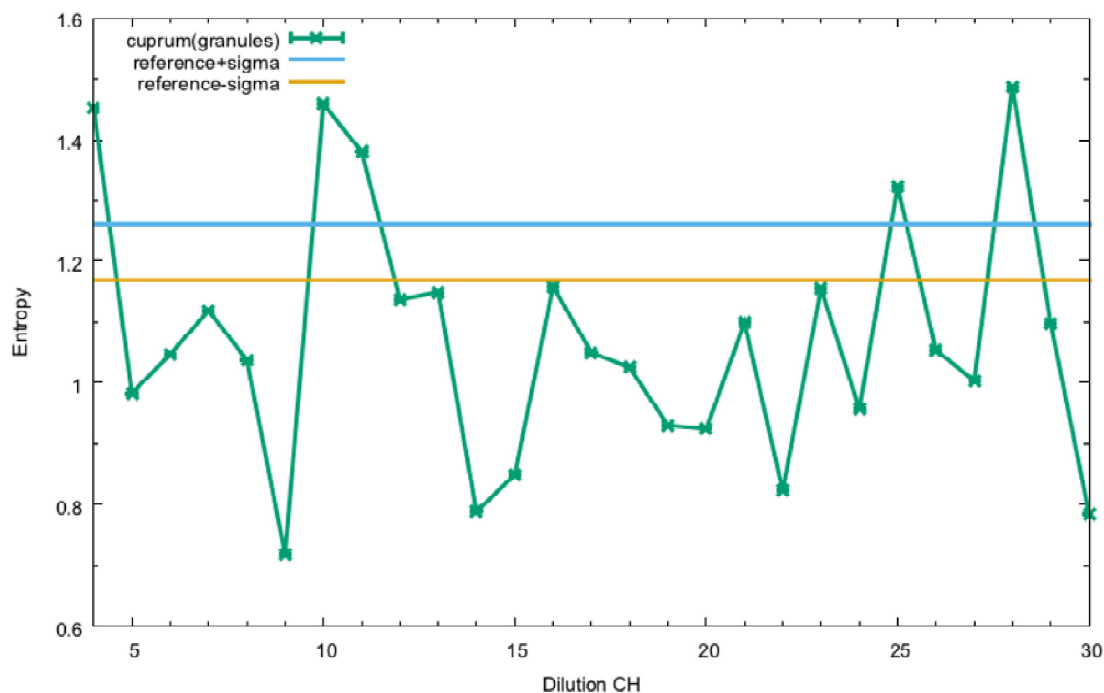


Figure 8. Variation in the entropy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised Cuprum metallicum solutions. The average entropy is $S = 1.074$ (0.219), with a skewness of 0.41 and a kurtosis of -0.59 .

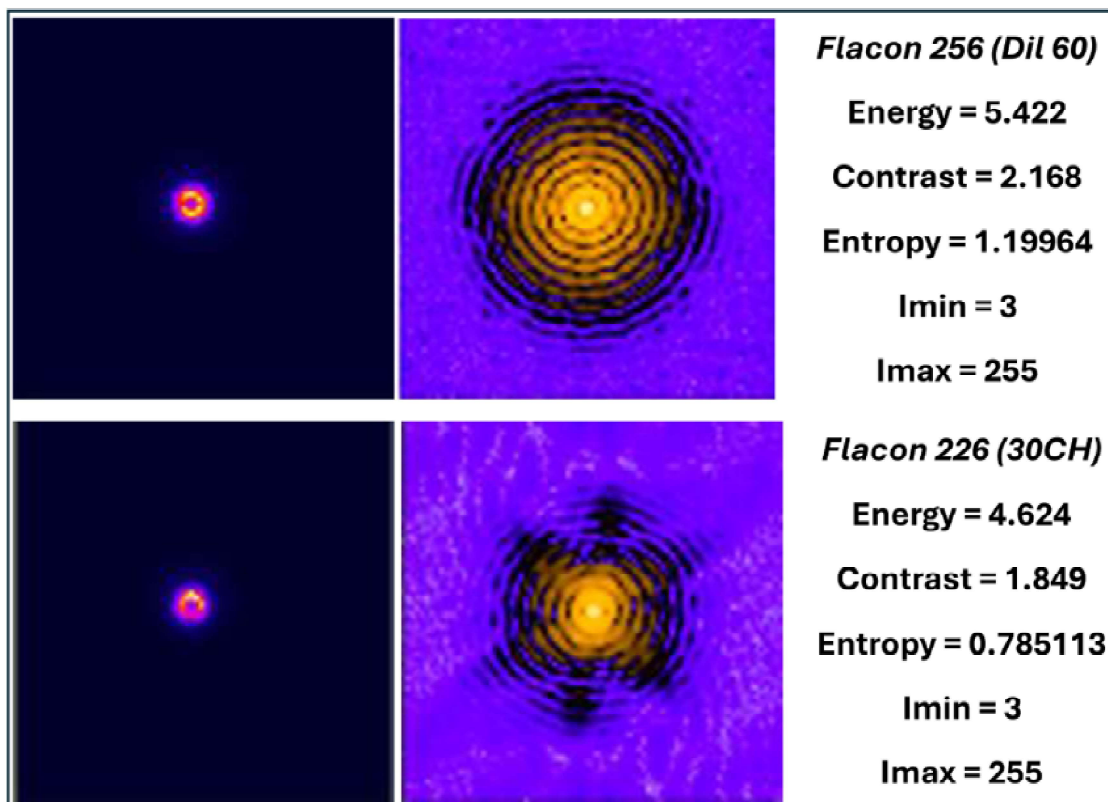


Figure 9. Coloured electrophotonic images with their fast Fourier transform for one dilution without dynamisation (DIL) and one dynamised (CH) Cuprum metallicum solution.

3.4. *Cuprum Metallicum* Size-6 Pilules (Korsakov)

Figure 10 shows representative electrophotonic images recorded on size-6 pilules impregnated with a dynamised *Cuprum metallicum* preparation at the 200 K level. The upper image refers to the preparation made in 2016 for this study, whereas the lower image corresponds to a preparation made approximately 30 years earlier by the same pharmacy. Within these illustrative observations, ageing did not appear to reduce the EPA-derived photonic energy or contrast. The aged sample also showed higher emission intensity and entropy than the fresh preparation. These differences may reflect changes in EPA-derived image texture or signal heterogeneity between the two preparations, but they should not be interpreted as evidence of long-term stability, preservation of preparation quality, or increased information content. The FFT image suggested a shorter apparent period, corresponding to higher spatial-frequency components in the analysed image features. These observations remain exploratory and require confirmation using independent aged and freshly prepared samples.

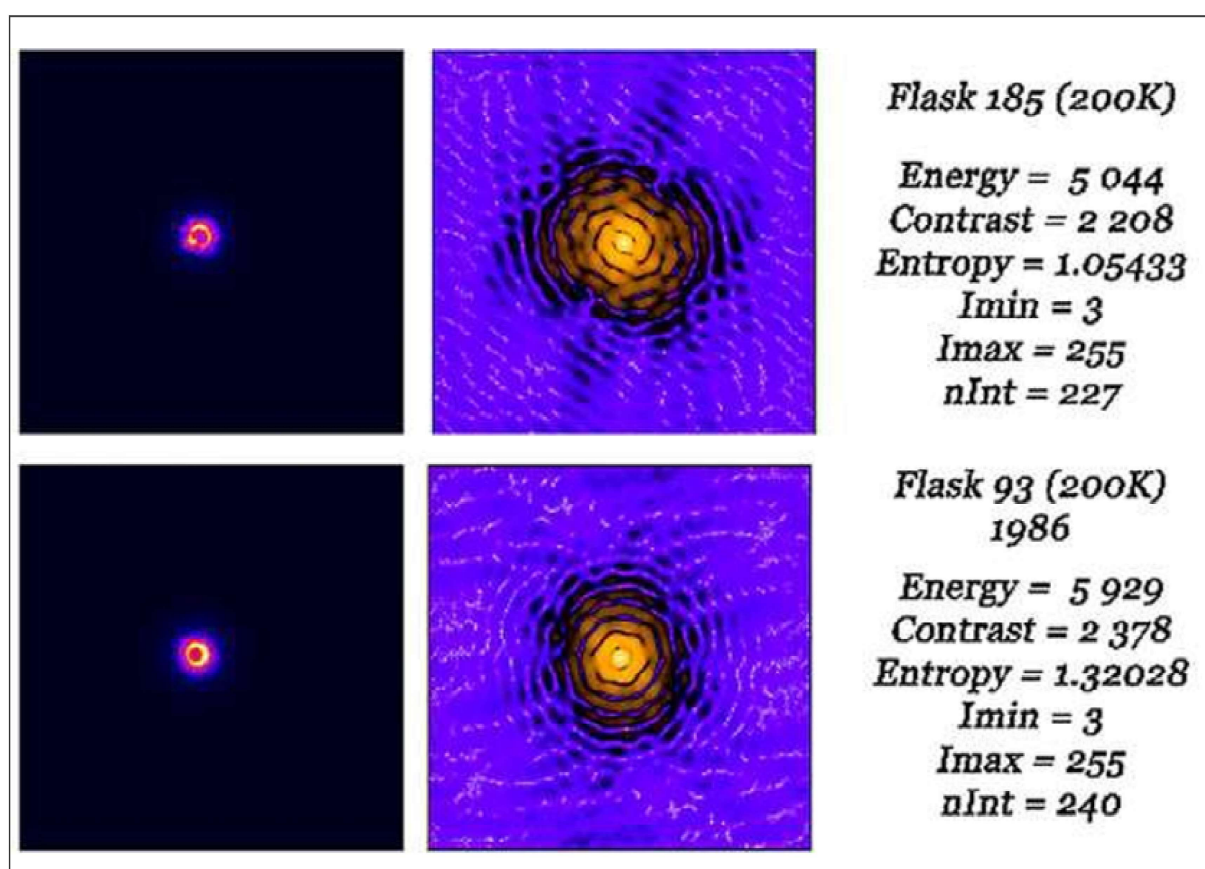


Figure 10. Electrophotonic images with their fast Fourier transform for two potentised *Cuprum metallicum* preparations made in 2016 (**top**) and 1986 (**bottom**) using the Korsakov method.

Figure 11 shows the total energy variations for size-6 pilules impregnated with dynamised *Cuprum metallicum* solutions prepared using the Korsakov method. Owing to the relatively large standard deviation, the average energy value did not clearly differ from the solvent-impregnated reference at the descriptive one-standard-deviation level. At the individual-sample level, most size-6 pilules showed energy values above the reference mean $\pm$ one standard deviation range, with the exception of the 6 K sample, which remained within the reference range. Compared with the Hahnemannian series, the distribution showed positive skewness relative to a normal distribution, suggesting lower energy values at higher dilutions within this dataset. The large positive kurtosis indicates that

the central part of the distribution was less represented than the tails. These observations remain exploratory and should not be interpreted as validated discriminatory criteria.

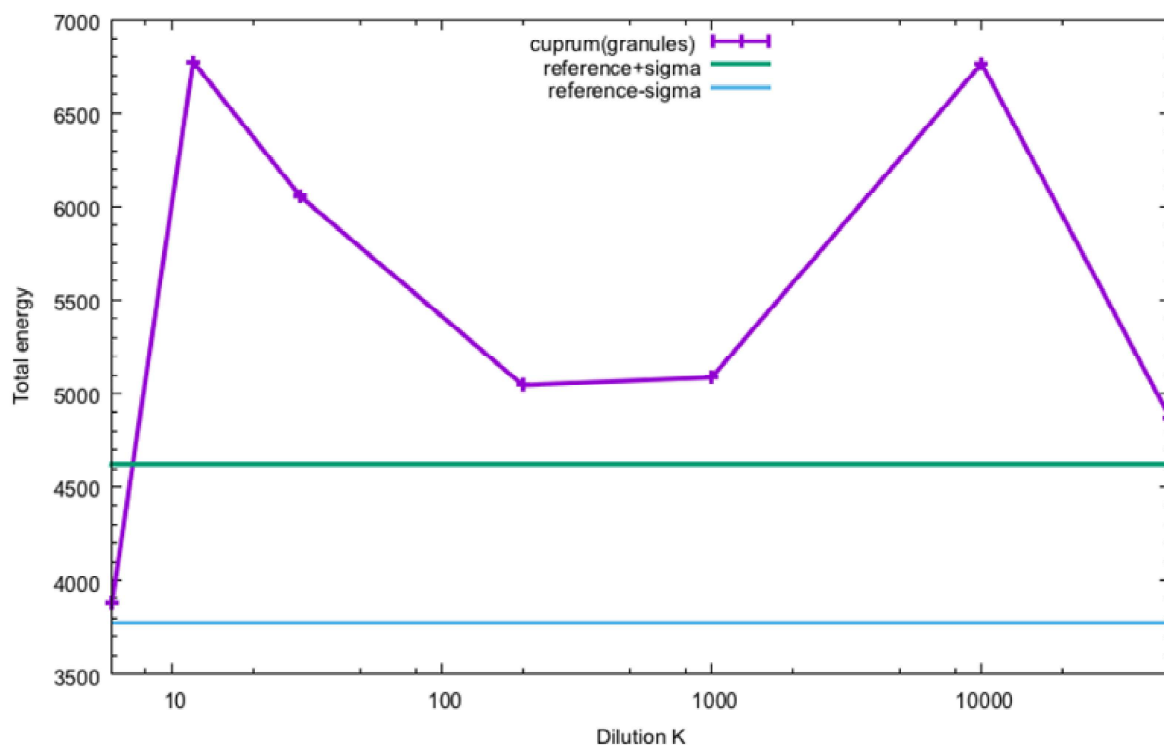


Figure 11. Variation in the total energy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised Cuprum metallicum solutions using the Korsakov method. The average energy value is $E = 5491$ (1067), with a skewness of 0.003 and a kurtosis of -1.18 .

Figure 12 shows the contrast variations for the same series of images. The average contrast value did not clearly differ from the reference at the descriptive one-standard-deviation level. It was also close to the value obtained using the Hahnemannian method. At the individual-sample level, only two size-6 pilules displayed contrast values within the reference mean $\pm$ one standard deviation range. As observed for energy, positive skewness relative to a normal distribution was observed, suggesting slightly higher contrast values at lower dilutions within this dataset. The large positive kurtosis indicates that the central part of the distribution was less represented than the tails, as also observed for total energy. These contrast-related observations should be interpreted as exploratory EPA-derived patterns and require confirmation in independent datasets.

Figure 13 shows the entropy variations for the same series of images. The average entropy value did not clearly differ from the reference or from the Hahnemannian method at the descriptive one-standard-deviation level. At the individual-sample level, three samples displayed entropy values outside the reference mean $\pm$ one standard deviation range, whereas the 200 K sample remained within this reference range. The distribution showed positive skewness relative to a normal distribution, suggesting higher entropy values at lower dilutions within this dataset. Positive kurtosis was also observed, indicating that the central part of the distribution was less represented than the tails, as observed for total energy and contrast. These entropy-related observations should be interpreted cautiously and should not be considered validated discriminatory features without independent confirmation.

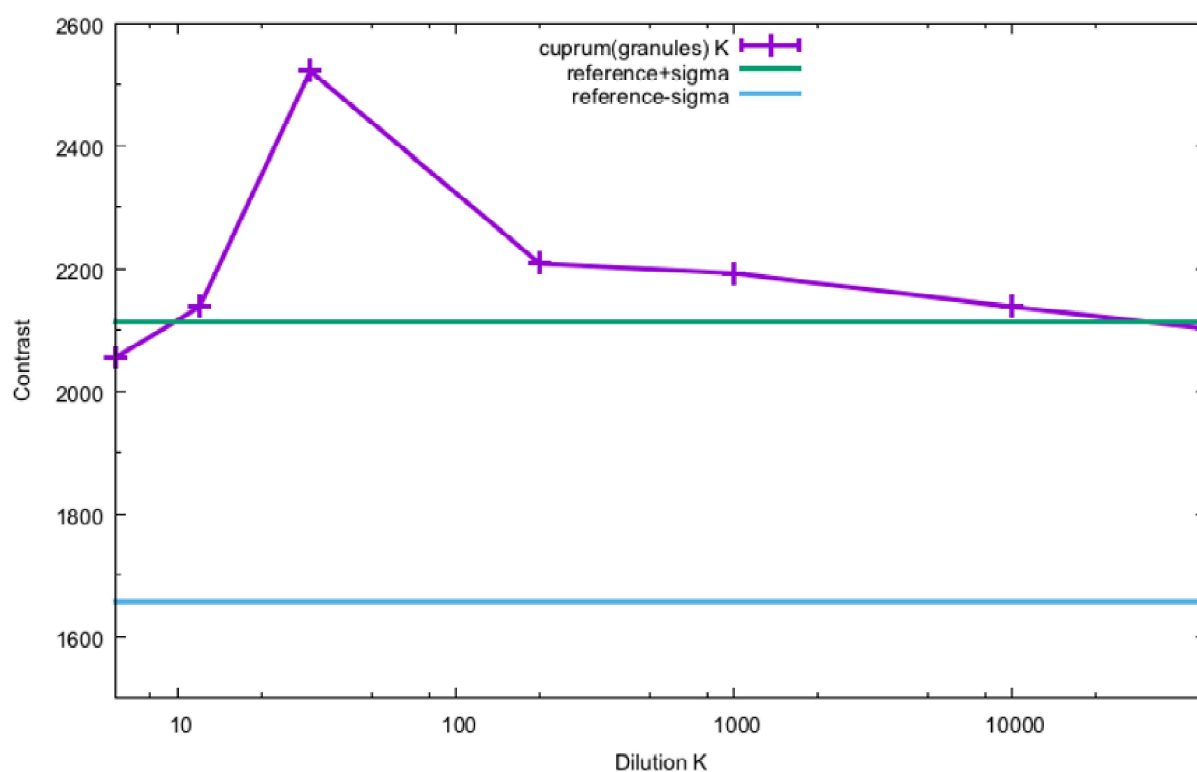


Figure 12. Variation in the total contrast for electrophotonic images recorded on size-6 pilules impregnated with various dynamised Cuprum metallicum solutions using the Korsakov method. The average contrast is $\Delta = 2123$ (80), with a skewness of 4.11 and a kurtosis of 17.4.

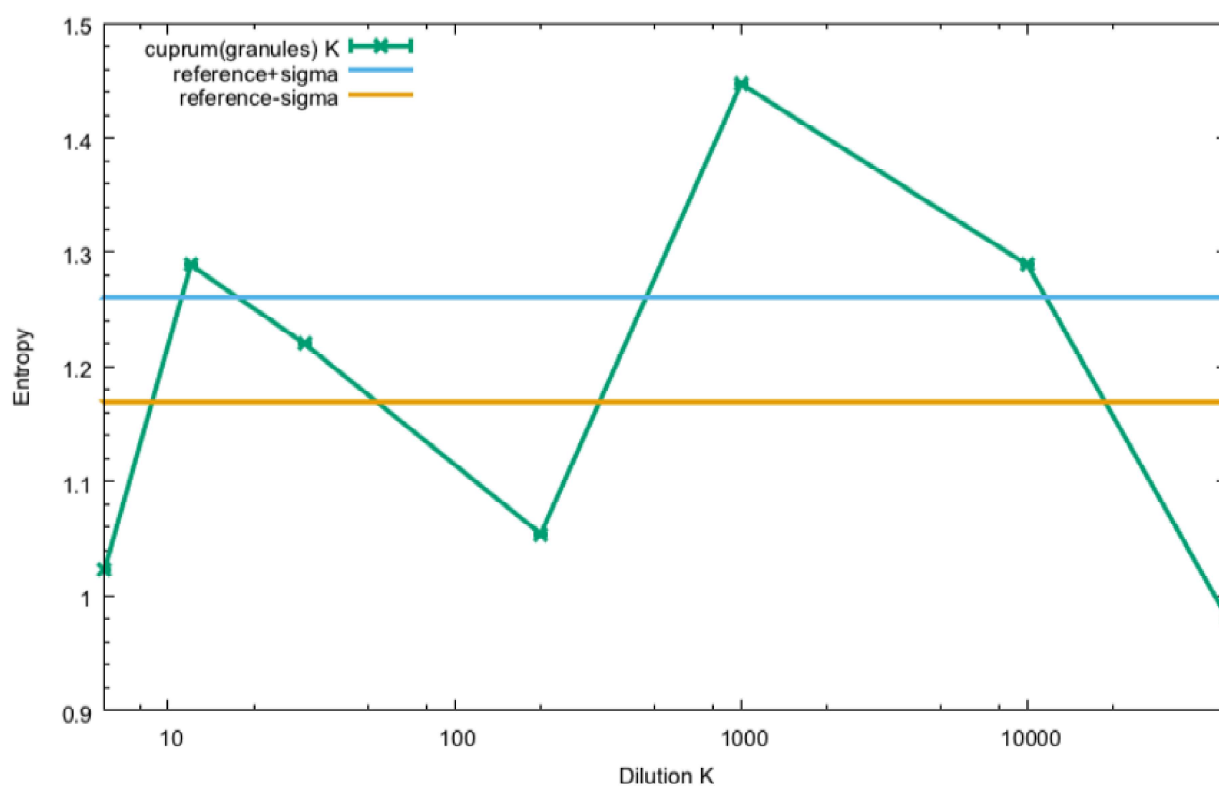


Figure 13. Variation in the entropy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised Cuprum metallicum solutions using the Korsakov method. The average entropy is $S = 1.028$ (0.118), with a skewness of 2.3 and a kurtosis of 4.2.

3.5. *Gelsemium* Size-6 Pilules (Hahnemann)

Figure 14 compares the electrophotonic images recorded on size-6 pilules impregnated with two simply diluted *Gelsemium* solutions, without dynamisation, with those recorded on size-6 pilules impregnated with corresponding dynamised solutions. As with the *Cuprum metallicum* samples, the dynamised samples showed lower energy, contrast and entropy values than the simply diluted samples within this dataset. These differences should be interpreted descriptively and require confirmation before they can be considered robust effects of the dynamisation process.

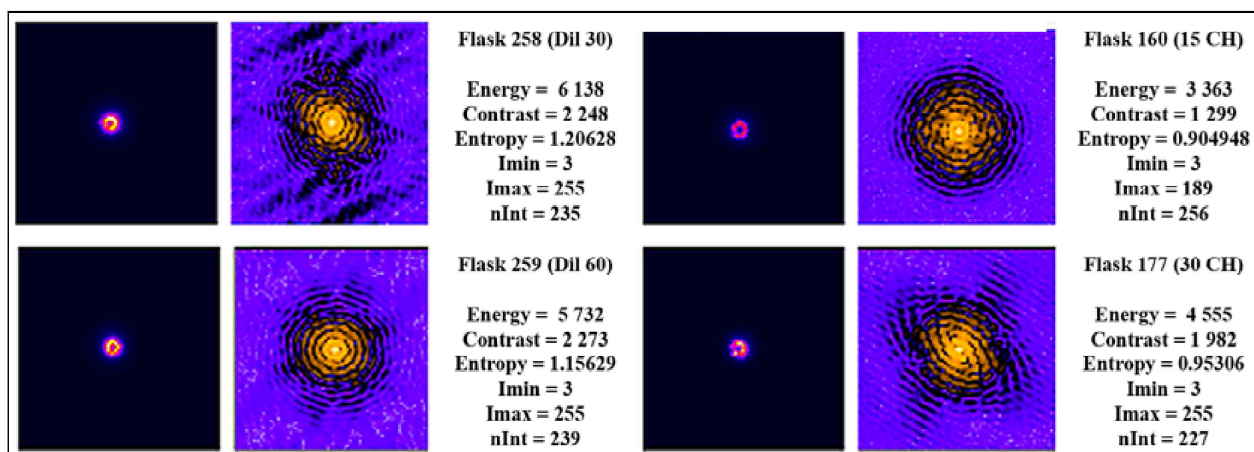


Figure 14. Electrophotonic images with their fast Fourier transform for two dilutions without dynamisation (left) and two dynamised (right) *Gelsemium* solutions.

Figure 15 shows the total energy variations for size-6 pilules impregnated with dynamised *Gelsemium* solutions. Owing to the relatively large standard deviation, the average energy value did not clearly differ from the pure-solvent reference at the descriptive one-standard-deviation level. At the individual-sample level, most size-6 pilules displayed energy values outside the reference mean $\pm$ one standard deviation range, except for the 11 CH, 23 CH and 24 CH samples, which remained within this reference range. The 7 CH sample showed a higher energy value than the other dilutions in this series. The distribution showed positive skewness relative to a normal distribution, suggesting higher energy values at lower dilutions within this dataset. Positive kurtosis was also observed, indicating that the central part of the distribution was less represented than the tails. Compared with the corresponding *Cuprum metallicum* series, the *Gelsemium* series showed a different descriptive EPA-derived profile, with energy values both above and below the reference range. A comparison of the *Cuprum* and *Gelsemium* image examples at the same dilution illustrates this. (Figure 16). These observations remain exploratory and require confirmation in independent datasets.

Figure 17 shows the contrast variations for the same series of images. The average contrast value did not clearly differ from the reference at the descriptive one-standard-deviation level. It also differed descriptively from that obtained for the *Cuprum metallicum* samples. At the individual-sample level, most size-6 pilules displayed contrast values outside the reference mean $\pm$ one standard deviation range. As observed for energy, the *Gelsemium* samples showed a different descriptive profile from the *Cuprum metallicum* samples within this dataset. In contrast to the energy distribution, negative skewness relative to a normal distribution was observed, suggesting higher contrast values at higher dilutions. Negative kurtosis was also observed, indicating that the tails of the distribution were less represented than the central range. These contrast-related observations should be interpreted as exploratory image-analysis findings rather than validated discriminatory markers.

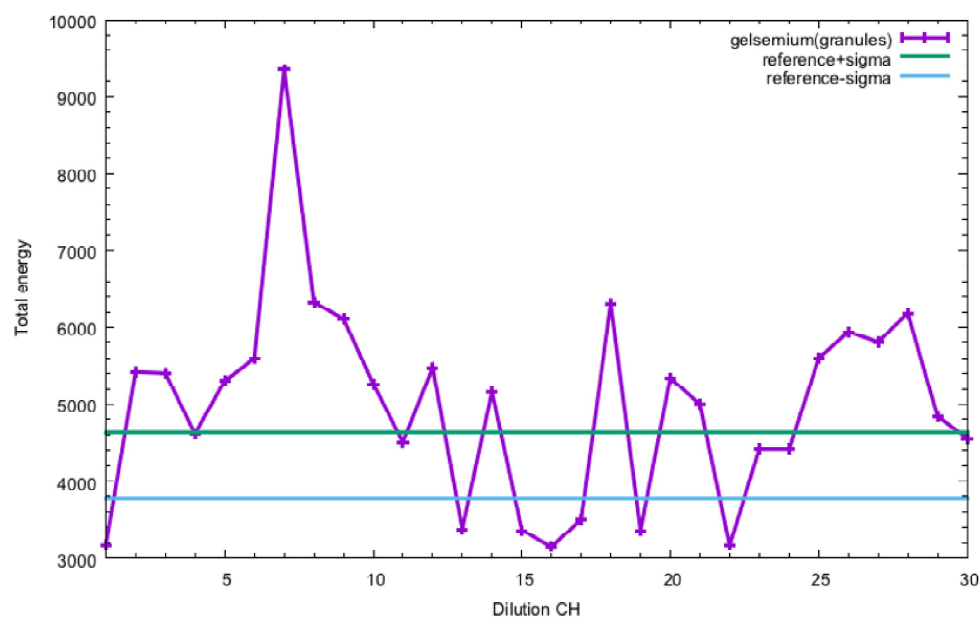


Figure 15. Variation in the total energy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised *Gelsemium* solutions. The average energy value is $E = 5001$ (1311), with a skewness of 0.83 and a kurtosis of 1.86.

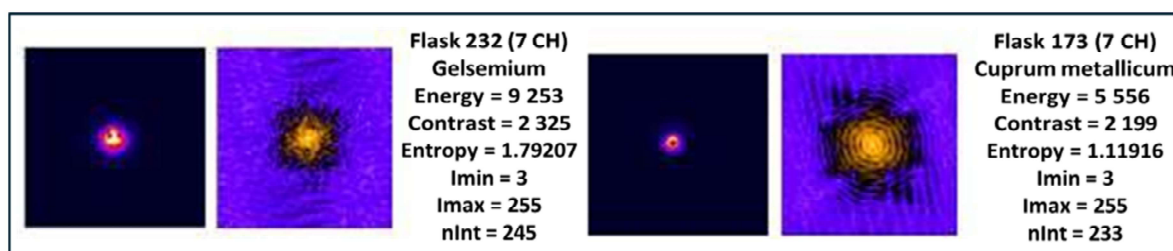


Figure 16. Comparison of electrophotonic images recorded for two samples, *Gelsemium* (left) and *Cuprum metallicum* (right), at the same dilution/dynamisation level.

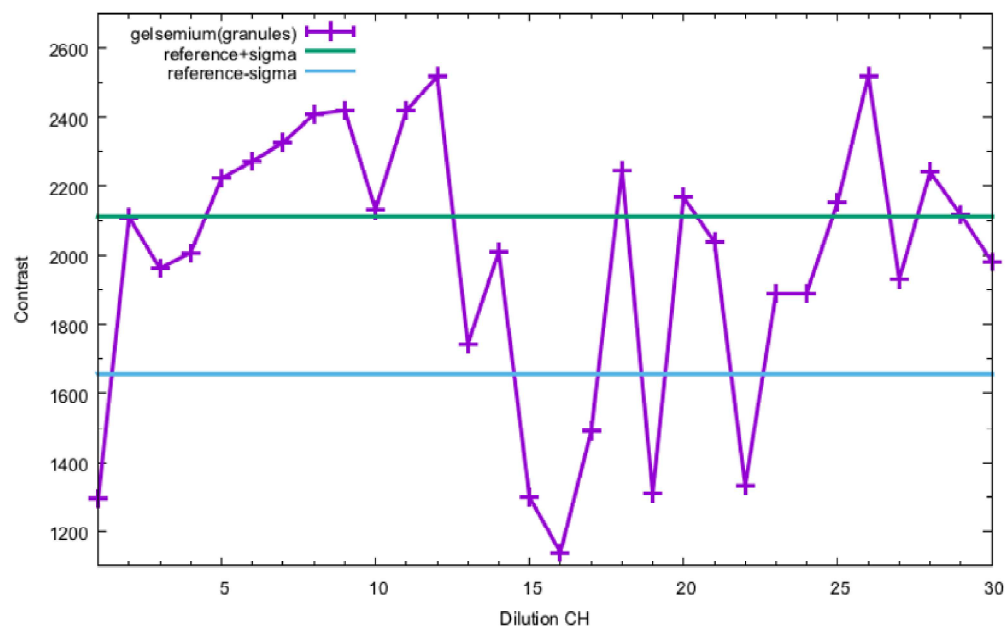


Figure 17. Variation in the total contrast for electrophotonic images recorded on size-6 pilules impregnated with various dynamised *Gelsemium* solutions. The average contrast is $\Delta = 1986$ (394), with a skewness of -0.72 and a kurtosis of -0.63 .

Figure 18 shows the entropy variations for the same series of images. The average entropy value did not clearly differ from the reference at the descriptive one-standard-deviation level. At the individual-sample level, entropy values were frequently outside the reference mean $\pm$ one standard deviation range, with some samples above and others below this range. As for *Cuprum metallicum*, the distribution showed positive skewness relative to a normal distribution, suggesting higher entropy values at lower dilutions within this dataset. Positive kurtosis was also observed, indicating that the central part of the distribution was less represented than the tails. These entropy-related patterns should be considered exploratory EPA-derived observations requiring independent confirmation.

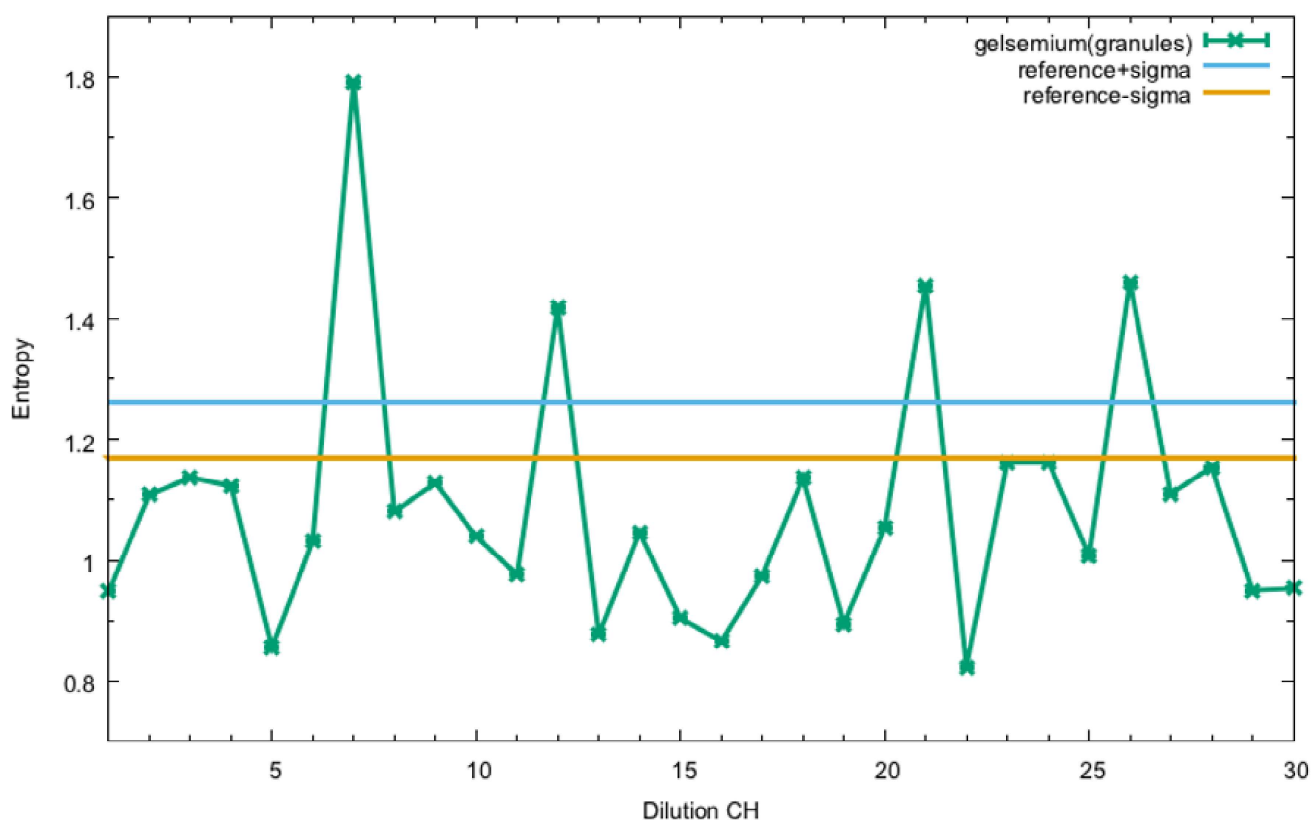


Figure 18. Variation in the entropy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised *Gelsemium* solutions. The average entropy is $S = 1.086$ (0.211), with a skewness of 1.47 and a kurtosis of 2.26.

3.6. *Gelsemium* Size-6 Pilules (Korsakov)

Figure 19 shows representative electrophotonic images recorded on size-6 pilules impregnated with dynamised *Gelsemium* solutions at the 200 K level. The image on the left refers to the preparation made in 2016 for this study, whereas the second image corresponds to a preparation made 13 years earlier by the same pharmacy. In this illustrative comparison, the aged sample showed lower EPA-derived photonic energy, contrast and entropy than the fresh preparation. These differences may reflect changes in EPA-derived image texture or signal heterogeneity between the two preparations, but they should not be interpreted as evidence for a systematic time-dependent decrease in information content or preparation quality. The FFT image suggested a larger apparent period and reduced spatial-frequency spreading in the analysed image features. These observations remain exploratory and require confirmation using independent aged and freshly prepared samples.

Figure 20 shows the total energy variations for size-6 pilules impregnated with dynamised *Gelsemium* solutions using the Korsakov method. The average energy value was

above the pure-solvent reference at the descriptive one-standard-deviation level. At the individual-sample level, the measured values in this small series were above the reference mean $\pm$ one standard deviation range. Compared with *Cuprum metallicum*, the distribution showed a more positive skewness and kurtosis relative to a normal distribution. These observations should be interpreted as descriptive EPA-derived patterns within this dataset and not as validated discriminatory criteria.

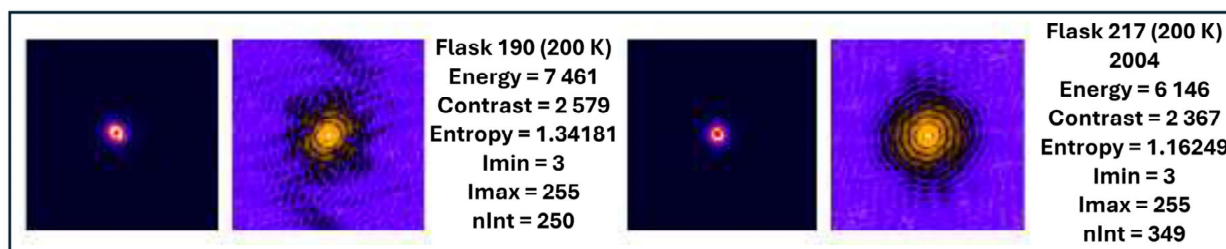


Figure 19. Electrophotonic images with their fast Fourier transform for two potentised *Gelsemium* preparations made in 2016 (left) and 2004 (right) using the Korsakov method.

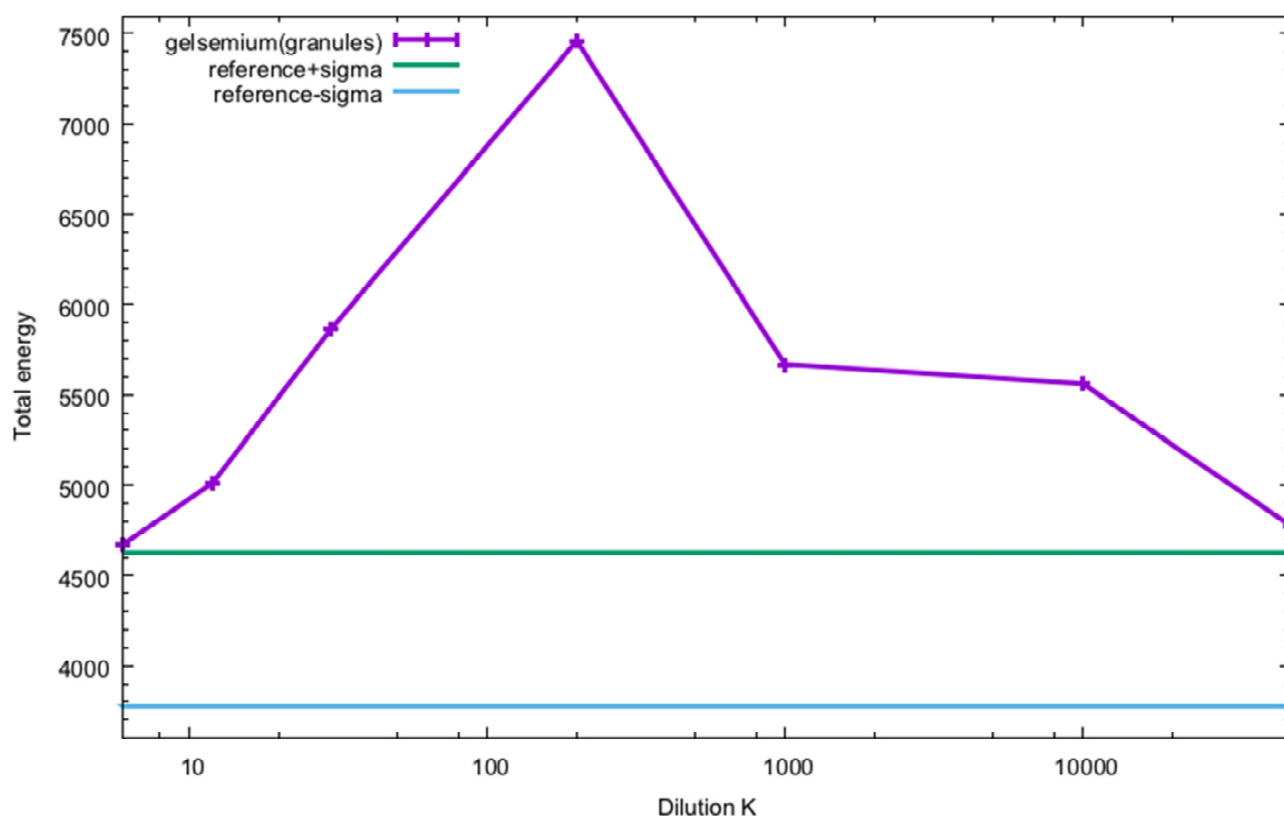


Figure 20. Variation in the total energy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised *Gelsemium* solutions using the Korsakov method. The average energy value is $E = 4958$ (553), with a skewness of 3.31 and a kurtosis of 11.34.

Figure 21 shows contrast variations for the same series of images. The average contrast value did not clearly differ from the reference at the descriptive one-standard-deviation level. It also showed a descriptive difference from the values obtained using the Hahnemannian method. At the individual-sample level, three size-6 pilules displayed contrast values within the reference mean $\pm$ one standard deviation range, whereas four size-6 pilules displayed higher contrast values. No measured value in this small series was below the reference mean minus one standard deviation. As for the energy distribution, positive skewness relative to a normal distribution was observed, suggesting slightly higher contrast

values at lower dilutions within this dataset. The large positive kurtosis indicates that the central part of the distribution was less represented than the tails. The curve also showed a different descriptive profile from that recorded for preparations manufactured using the Hahnemannian method. These findings remain exploratory and require confirmation in independent datasets.

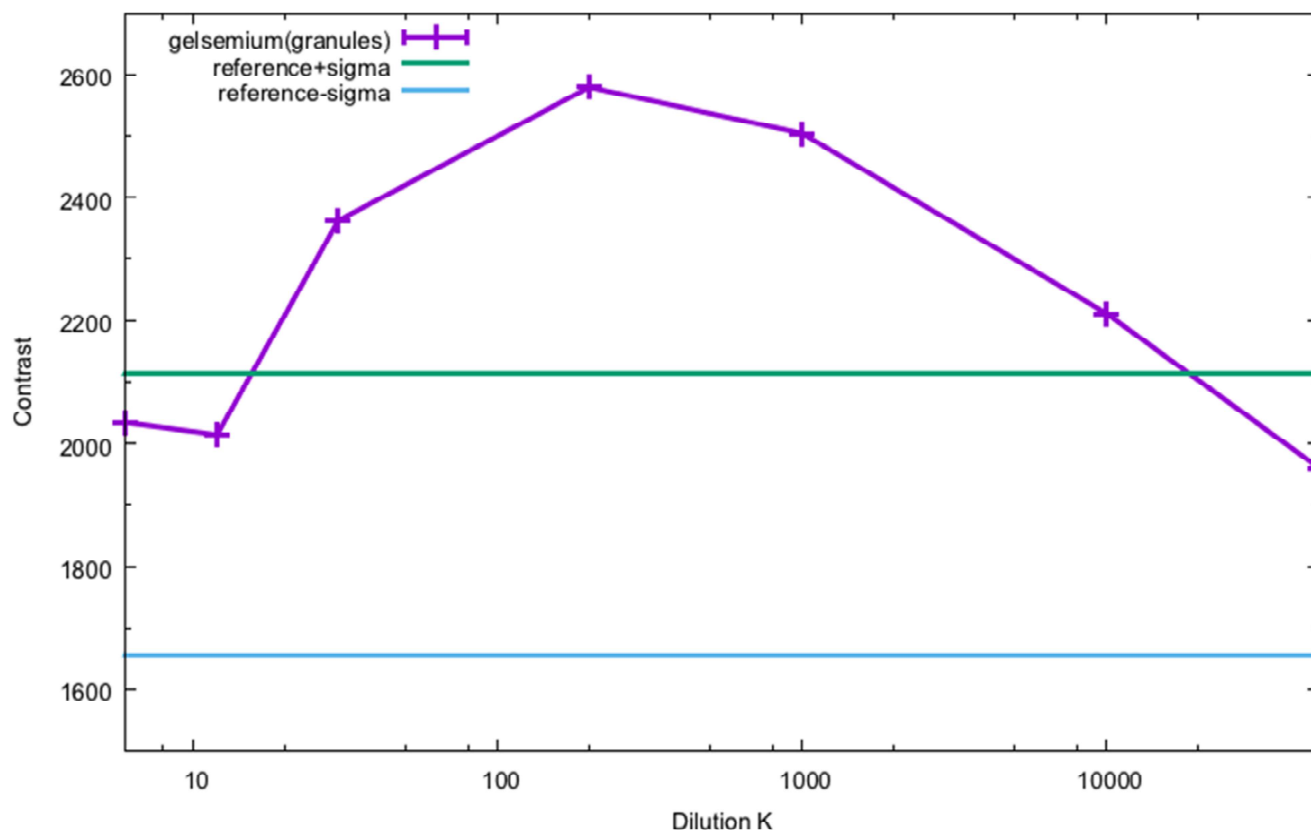


Figure 21. Variation in the total contrast for electrophotonic images recorded on size-6 pilules impregnated with various dynamised *Gelsemium* solutions using the Korsakov method. The average contrast is $\Delta = 2024$ (165), with a skewness of 2.38 and a kurtosis of 4.34.

Figure 22 shows the entropy variations for the same series of images. The average entropy value did not clearly differ from the reference or from the Hahnemannian method at the descriptive one-standard-deviation level. At the individual-sample level, entropy values were often outside the reference mean $\pm$ one standard deviation range, either above or below this range. The distribution showed positive skewness and kurtosis relative to a normal distribution, as also observed for the energy and contrast curves. The distribution also showed a different descriptive profile from that obtained with *Cuprum metallicum*. These entropy-related observations should be interpreted cautiously and require confirmation in independent datasets before they can be considered robust analytical features.

At this stage of the data analysis, the results suggest that size-6 pilules impregnated with dynamised *Cuprum metallicum* or *Gelsemium* preparations may show different EPA-derived image profiles under the experimental conditions used here. The Hahnemannian and Korsakovian protocols also appeared to be associated with different descriptive image patterns in this dataset. Comparisons involving aged samples suggested possible differences from freshly prepared or reference samples, but these observations should not be interpreted as evidence of systematic ageing effects. Much larger and independently replicated datasets would be required before any unbiased conclusion can be drawn. The electrophotonic images generally displayed a variably bright globular appearance, suggest-

ing that, under these acquisition conditions, the recorded response may have been mainly associated with the negative pulses of the generator.

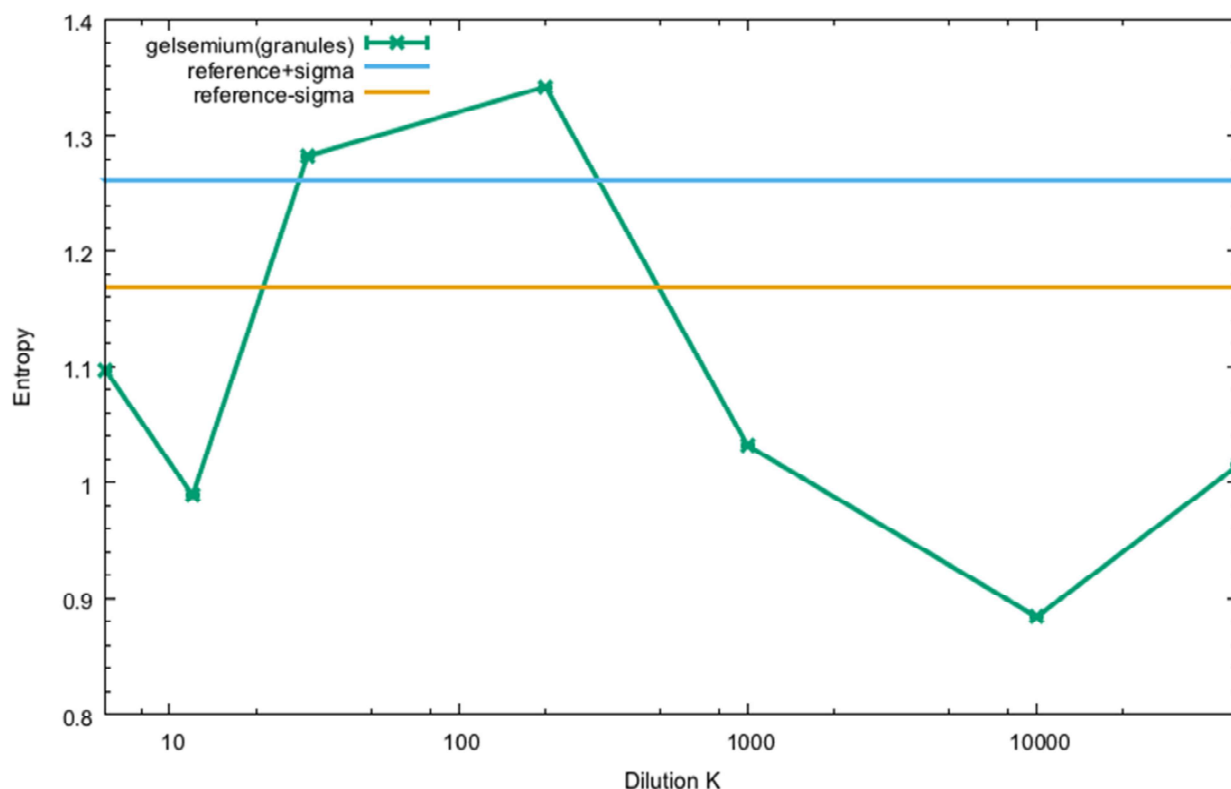


Figure 22. Variation in the entropy for electrophotonic images recorded on size-6 pilules impregnated with various dynamised *Gelsemium* solutions using the Korsakov method. The average entropy is $S = 1.033$ (0.082), with a skewness of 2.55 and a kurtosis of 7.11.

4. Discussion

The interpretation of these results must take into account possible variation in environmental parameters such as humidity, CO_2 concentration and temperature. Such variation could have contributed to fluctuations in the measurements. Although the measurements were performed under blinded conditions, in randomised order and on the same day, these precautions do not exclude environmental influences. The limited number of statistically significant differences should therefore be interpreted cautiously and regarded as preliminary observations requiring further confirmation.

The measurement conditions for the pilules were also monitored. Several physical properties can affect the recorded discharges. Surface hydration, ambient humidity and residual moisture content can alter surface conductivity and create preferential pathways for electron avalanches. Micro-geometry and roughness, including variations in the physical contact surface, micro-cracks or uneven curvature of individual pilules, can change local electric-field concentrations. Dielectric permittivity, which depends on the structural composition and packing density of the substrate material, typically lactose or sucrose bases, can modify local capacitance and charge accumulation under the applied nanosecond/high-frequency voltage pulses.

The validity of these findings may also be questioned in the absence of further independent verification using complementary analytical methods. Confirmation across a broader range of source materials and sample sets will be required. The present observations can be considered in the context of previous physicochemical studies of similar impregnating liquids using established analytical approaches, including NMR, NTA, SEM,

EDX, FTIR, pH measurements and chromatography; however, these earlier findings do not by themselves validate EPA as a discriminatory method.

Previous studies using nuclear magnetic relaxation times have suggested that liquid potentised preparations may differ from pure solvent and may display altered water-related relaxation properties [12–24]. Other studies have reported the presence of nanoparticles or nano-objects in highly diluted preparations [25–44]. In related work, each pilule was estimated to contain very small amounts of particulate or dry matter, with distributions and physicochemical profiles that may depend on the source material introduced at the start of the manufacturing process. Reported pH values also differed between *Gelsemium* and *Cuprum* preparations [45–48], and ion/molecular partition data suggested composition-related differences [49]. Figures 23–25 illustrate that, although elemental and molecular compositions of the preparations impregnated onto the pilules are broadly similar, they may differ in specific proportions and quantities [26,38,48,49]. These constituents are present at very low concentrations, and impregnation onto the pilules further reduces their amounts. For the *Cuprum* source material, lyophilisation of the solution used for impregnation yielded 30 µg of dry solid matter per gram of aqueous solution, whereas for *Gelsemium* the corresponding value was 42 µg/g; these values remained broadly consistent throughout the potentisation process. During pilule impregnation, the volume of liquid used represented 1% of the pilule weight. Since a batch of 80 pilules weighs 4 g, the amount of liquid used was therefore 0.04 g (40 mg), corresponding to 0.5 mg of solution per pilule. After drying, only dry matter remains within the pilules, corresponding approximately to 15 ng per pilule for *Cuprum* and 21 ng per pilule for *Gelsemium*. The main component of the dry matter was sodium bicarbonate [49]. These very small quantities may help explain why conventional analytical methods can be challenging in this context. The EPA observations reported here provide a rationale for further systematic and better-controlled measurements, but they should not be interpreted as evidence that EPA already provides validated discriminatory information. A substantial EPA image database, including numerous samples characterised by complementary techniques and assessed across progressively lower dilutions, will be required before firm conclusions can be drawn regarding the usefulness of this approach.

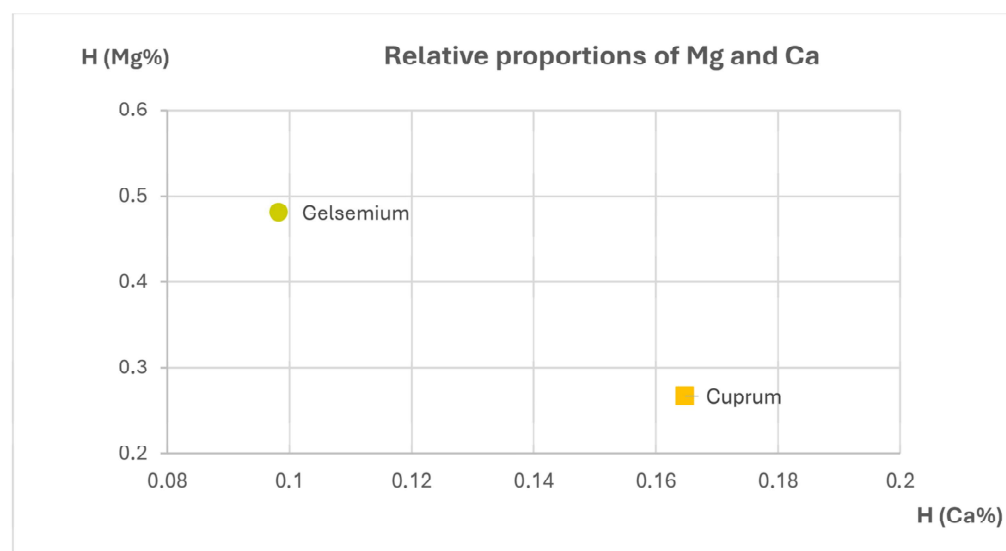


Figure 23. Representative differences in the average relative concentrations of magnesium and calcium, as determined by EDX analysis, in the impregnation liquids of *Gelsemium* and *Cuprum*. These findings are consistent with different ionic composition profiles in the preparations used for pilule impregnation.



Figure 24. Difference in average quantity of material collected from the impregnated solutions and freeze-dried for Gelsemium and Cuprum.

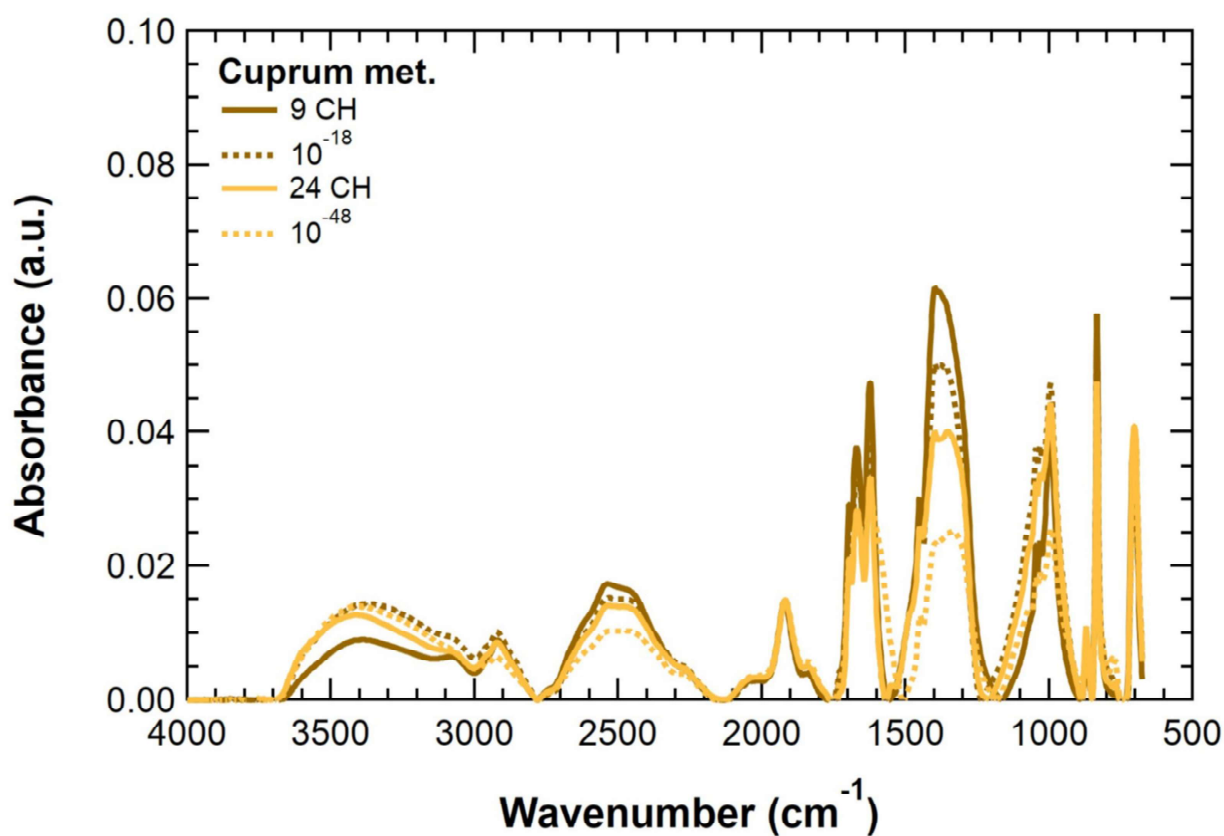


Figure 25. *Cont.*

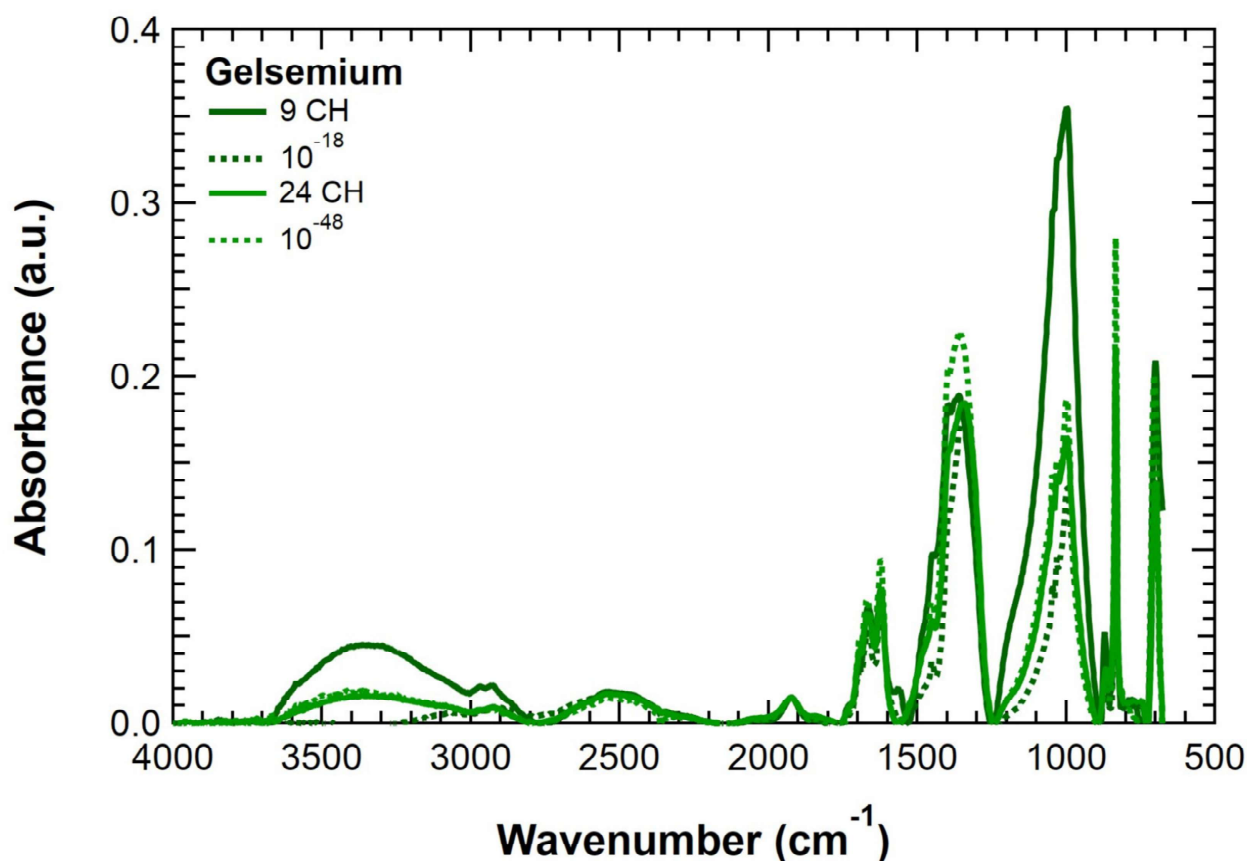


Figure 25. Descriptive comparison of molecular composition in *Cuprum* and *Gelsemium* preparations. Sodium bicarbonate was the main component detected, together with carbonates, alkanes and silicates, with different relative proportions in the two preparations.

The Droplet Evaporation Method (DEM) is a cost-effective and rapid approach for analysing liquid samples used for pilule impregnation. It involves studying the patterns formed during droplet evaporation and has been reported to reveal differences between some highly diluted preparations [50–55]. In parallel, EPA has recently been explored for liquid and dry potentised preparations [9,56]. At present, however, EPA should be regarded as an exploratory image-analysis approach rather than as an established analytical method for assessing potentised medicines.

Although EPA has recently undergone preliminary methodological evaluation, further work is needed before its analytical scope can be defined. Future studies should focus on the reproducibility of FFT-derived spatial-period and spatial-frequency indices obtained from different potentised preparations. Improvements in instrumentation, acquisition stability and spectral characterisation may help clarify whether these image-derived indices have robust analytical relevance.

The relationship between pixel intensity, FFT-derived spatial-period/spatial-frequency indices and the wavelengths of the UV emission remains uncertain. A relationship may exist, but the present results cannot be directly correlated with UV wavelengths. Although pixel intensity can be used to calculate image-derived energy (E), this parameter does not represent the energy of individual photons. Direct conclusions about wavelength would therefore require different experimental techniques and dedicated spectral measurements.

Specific nuclear magnetic relaxation times, nanoparticle-size distributions, ion and molecular partitions confirmed by SEM-EDX-FTIR, and pH-related differences cannot yet be directly linked with the EPA results. Nevertheless, the convergence of observations

across different approaches is compatible with the possibility of a relationship between these findings, although its nature remains to be established. This point should be regarded as a working hypothesis rather than as a demonstrated mechanism.

Because the manufacturing process is stepwise, each preparation stage depends on the preceding stage; this should be considered when interpreting serial measurements of potentised preparations.

A similar caution applies to the interpretation of spatial-frequency indices. Within this dataset, these indices showed relatively coherent profiles across successive dilution/dynamisation steps, with different distributions for Cuprum and Gelsemium. Comparable differences have been reported in nanoparticle tracking analysis (NTA), where the size distribution (SPAN) of copper-associated nano-objects was smaller than that observed for Gelsemium. The potential role of nanoparticles and nanobubbles therefore merits further investigation, but no causal link with the EPA-derived parameters can yet be established.

The limitations of this exploratory study should be acknowledged. Because only one independent production line was evaluated for each source material, it is not possible to statistically disentangle the effect of the starting material from that of the specific batch or preparation history. In practice, the “preparation history” is intrinsically linked to the nature of the substance itself. However, from the first common manufacturing step onwards (4 CH), the dilution and dynamisation procedures were identical. It might therefore be expected that any effects related to the raw material, or to differences arising during the first three stages of preparation, would gradually diminish and converge over subsequent preparation steps. This convergence, however, was not observed in the present dataset. Nevertheless, any definitive conclusion would require the analysis of multiple independent production lines.

Residual water and ethanol contents were not directly determined in the measured pilules. Although the samples were prepared using a standardised drying procedure and analysed under monitored, relatively stable laboratory conditions, equivalence of residual moisture between samples cannot be assumed. Given the known sensitivity of ionisation and surface conductivity to moisture, this should be considered a limitation of the present exploratory study. Future work should include direct measurement of residual water and ethanol contents.

Another limitation of this study is that, although statistically significant differences were observed between the two complete manufacturing lines (Cuprum versus Gelsemium), the present design cannot disentangle the specific contribution of individual preparation steps, such as the preliminary solid trituration required for insoluble materials, from the effect of the raw material itself. A complete serial blank production line, for example, lactose triturated and diluted in series without active source material, would be required to isolate these factors mechanistically. Nevertheless, comparing complete production lines remains relevant because it reflects the real-world manufacturing protocols dictated by official pharmacopoeias.

Although image acquisition and primary image processing were performed under blinded conditions, this should not be interpreted as validation of EPA as a definitive discriminatory or classification method. Blinding ensured that sample identity was concealed during measurement and analysis, thereby reducing observer-related bias. However, the present exploratory study did not include an independent validation set, predefined classification rules, or prospective testing of unknown samples. The observed differences in EPA- and FFT-derived parameters should therefore be interpreted as preliminary and hypothesis-generating. Further studies using larger sample sets, independent production lines, external validation samples and predefined decision criteria are required before EPA can be considered a reliable method for classifying unknown preparations.

5. Materials and Methods

Electroluminescence, i.e., the emission of light from condensed matter under electrical excitation, has been investigated in organic molecular solids since the 1950s, notably in the work of Pope et al. and Helfrich et al. [57–60]. Electroluminescence is an optoelectronic phenomenon in which a material emits light in response to the passage of an electric current or exposure to a strong electric field. Electrons bound in atoms, molecules, and solids occupy discrete states characterised by well-defined binding energies. When an external source provides energy exceeding the binding energy of a given electron, that electron may be emitted into free space with an excess kinetic energy equal to $h\nu - E_B$. The resulting kinetic-energy distribution therefore reflects the distribution of electron binding energies in the atomic, molecular, or crystalline system: an electron emitted from a state of binding energy E_B is observed with kinetic energy $E_k = h\nu - E_B$. When the external excitation is removed, the electron returns to a lower-energy state, releasing the excess energy in the form of light. Detailed references are provided in the article validating the method, published in the University of Florence's *Journal of Physical Chemistry* [9].

Table 1 summarises the experimental conditions under which the images were acquired. All measurements were taken on a single day in an air-conditioned room, with measured variations in temperature and humidity between morning and evening. Because the measurements were performed in randomised order with mixed samples, these variables are less likely to explain systematic differences across the series; however, their influence cannot be fully excluded.

After acquisition, greyscale images were analysed using three parameters based on the intensity $f(i, j)$ for each pixel (i, j) : the L2-norm, which corresponds to the total energy E of the image:

$$E = \sqrt{\sum_{i=1}^n \sum_{j=1}^n f(i, j)^2}$$

- The H1 norm corresponds to the total contrast Δ between neighbouring pixels:

$$\Delta = \sqrt{\sum_{i=1}^n \sum_{j=1}^n [f(i, j) - f(i-1, j+1)]^2 + [f(i, j) - f(i, j+1)]^2 + [f(i, j) - f(i+1, j+1)]^2 + [f(i, j) - f(i-1, j)]^2 + [f(i, j) - f(i+1, j)]^2 + [f(i, j) - f(i-1, j-1)]^2 + [f(i, j) - f(i, j-1)]^2 + [f(i, j) - f(i+1, j-1)]^2}$$

- Shannon's entropy (S) is the measure of the total information contained in the image:

$$S = -\sum_{i=1}^N p_i \times \log_2 p_i$$

where p_i is the probability of the occurrence of intensity i among the $N = 256$ possible greyscale values. For a pure white or black image, $S = 0$ (there is no information), while for a purely random image ($p_i = 1/256$), $S = \log_2(256) = 8$ (maximum entropy or information). For different samples, three images were captured.

5.1. The Method Used to Decipher Images

Fast Fourier transform was used to identify the ultraviolet period/spatial-frequency distribution in the different samples. It is important to note that the FFT of an image provides spatial periods (the inverse of spatial frequency) and spatial frequencies (cycles per pixel), not the wavelength of light directly. Pixel intensity does not correlate with wavelength, but rather with flux or photon quantity. Great care must therefore be taken when attempting to correlate these data with ultraviolet (UV) wavelengths. The energy of

a photon is related to its wavelength; however, pixel intensity (E) can only be cautiously and indirectly considered in relation to wavelength. Because the camera captures only UV light, the FFT images may be very cautiously and approximately related to UV light wavelengths. The raw experimental photographs were captured in a 16-bit greyscale format (0–65,535 grey levels) to maximise pixel intensity resolution in the spatial domain. For texture-frequency analysis, a Fast Fourier Transform (FFT) was computed using FIJI software (ImageJ version 1.52/framework version 2.0.0, NIH, Bethesda, MD, USA). During this operation, FIJI automatically scales the large dynamic range of the raw Fourier power spectrum using a logarithmic transformation, outputting the final FFT image in an 8-bit greyscale format (0–255 grey levels, $I_{max} = 255$). All subsequent quantitative operations in the frequency domain, such as threshold-based cluster segmentation and the calculation of spatial entropy, were carried out on these 8-bit FFT images.

5.2. Samples Manufacturing Process

Materials. Brown pharmaceutical 15 mL soda-lime-silicate glass flasks (ISO 719:2016, ISO 4802-1:2016, Ph. Eur. 3.2.1.; USP 39–NF 34: ≤ 0.85 mL 0.02 N HCl/g) were used [61]. They were closed with tight plastic caps using an under-screwed polypropylene closure system (Aceso[®] PPM H250 grade). For K potencies, the K-Tronic machine from Labotics[®] laboratory (2660 Hoboken Belgium) was used.

Vehicles and solvent. Purified water (deionised water) was obtained from a Millipore Milli-RX 45 (MERCK MILLIPORE, 67120 Molsheim, France) purification apparatus, serial number FSDM 96292D. Ethanol 62% m/m was prepared from ethanol 96% v/v, Certa Ph. Eur., lot 15E07-C01-16614 P, dated 09/07/2015, expiry 05/2018, UN1170, authorisation no. 597R01872. Lactose monohydrate, moderately fine, was obtained from Fagron (7864 Lessines, Belgium), 1 kg, authorisation no. 846IR05297, Ph. Eur., lot 15D10, expiry 02/2017. Pilules were GRANUSORB 4S Labotics (2660 Hoboken, Belgium) inert substances for homoeopathic preparations, reference 18411, 10/2015, lot no. 15050154010, 25 kg, expiry 08/2020. Composition: 100% sucrose; diameter: 4 mm; 20 pilules per gram.

Stocks: Stocks (source material) are substances, products or preparations used as starting materials, as mother tinctures for raw materials of botanical origin, or the substance itself for raw materials of mineral origin. Cuprum metallicum powder, Sigma, 50 gr. 203122, lot #MKBS 4830, Pcode 1001 89 73 74, containing 99,999% trace metals provided by Labotics Company. Gelsemium sempervirens MT HAB 65% v/v 500 mL Heel Belgium 982 UH 911 134 lot548463 Exp 12 2018.

Dilution/dynamisation process(es) or potentisation. All samples and controls were made under a validated (ISO 14644-1:2016) laminar flow in the Pharmacie Delvenne laboratory (5000 Namur Belgium). The *European Pharmacopoeia* [61], which describes precisely how the manufacturing process must be done, was strictly followed (GPP-compliant laboratory. Belgian Royal Decree, 2009). The pharmacist's equipment included mask (possible toxic effect on respiratory tract), protective glasses (possible irritating effect of emanations during crushing process, for example), shoe protection, a white apron, no perfume, a head cap and gloves. Hands were washed meticulously before carrying out any operation.

Dilutions and triturations are obtained from stocks by a process of centesimal potentisation in accordance with a homoeopathic manufacturing procedure: this means successive dilutions and succussions, or successive appropriate triturations, or a combination of the 2 processes. The number of potentisation steps defines the degree of dilution; for example, “3 cH” (Hahnemannian) or 3 K (Korsakovian) means 3 centesimal potentisation steps.

Difference between these two potentisation techniques. Use of vials/Hahnemannian: A new vial is used at each dilution stage (separate-vial method). Korsakovian: A single vial is reused throughout the entire process (single-vial method). Dilution ratio/Hahnemannian:

Precise and constant, generally 1:100 (Hahnemannian Centesimal—CH) or 1:10 (Hahnemannian Decimal—DH). For a CH dilution, 1 part of the substance is mixed with 99 parts of solvent. Korsakovian: Controlled and based on the principle of “residual adherence”. The vial is emptied by a burst of air directed at the liquid, which is thus forced out of the vial. The amount of liquid that remains clinging to the walls is 1 part, to which 99 parts of solvent are added to obtain a Korsakovian dilution (K). The dynamisation process is the same for both CH and K dilutions: 50 dynamisations per second for 2 s (100 ± 1 dyn) (Labotics validated Dynamiser).

For Gelsemium mother tincture, all dilutions, whether dynamised or simply diluted, were performed in ethanol 62% *v/v*. Deionised water was drawn directly from the tap after an initial flushing step. Between water samplings, the tap tip of the water bottle was kept in a small bottle of alcohol to avoid ambient contamination. Before each sampling, water was allowed to run into the sink to rinse the tip. Gilson PipetmanP (Gilson Benelux 2495A.J. The Hague, The Netherlands) micropipettes (P200: 50 to 200 μ L and P1000: 200 to 1000 μ L), fitted with suitable single-use tips, were used to collect samples. All steps were performed in new 15 mL glass containers after washing and high-temperature drying. The dynamisation process was performed (100 ± 1 shocks in 2 s) using a certified and validated Labotics© device, Dynamat©. Simple dilutions, used as complementary controls, were prepared in the same way but without the dynamisation process. A simple, gentle manual rotary mixing process was carried out by turning the bottle upside down.

For insoluble source materials: Cuprum metallicum, the first three triturations were performed manually following the standardised rules and controls of good pharmaceutical practice (GPP). Monohydrated, moderately fine lactose was used. Then the subsequent dilutions were made with ethanol 62% *v/v* as described above. The simply diluted controls were prepared following the same successive steps after 3 triturations. Further dilutions were prepared in ethanol 62% *m/v/v* but without the dynamisation process.

K potencies were prepared in a single vial using the K-Tronic© Labotics machine. Each dilution was made at a ratio of 1:100, dynamised with 100 succussions over 2 s, and then the vial was emptied using pulsed air. The first liquid potency was then placed in the vial. For Cuprum, this was the 4C aqueous potentisation, and for Gelsemium, it was the first 1C potentisation prepared with 62% *m/m* ethanol. The machine-prepared potentisations were aqueous. We took the intermediate potentisations that we wished to keep and diluted them once more with 62% *m/m* ethanol to preserve them for impregnating the size-6 pilules.

For example: We take the 199 K aqueous potentisation from a 30 mL bottle, empty it, and then dilute it with 99% ethanol relative to the remaining weight of the dilution on the bottle’s walls, thus obtaining 200 K. We retain the 6, 12, 30, 200, M, XM and LMK potencies. K potencies have since been described in Ph. Eur. 11.5–2371.

The manufacturer of size-6 pilules is required to comply with the “Excessive residual moisture” Pharmacopoeia. The relevant monograph specifies strict quality criteria, including mass uniformity, which implies consistent pilule size and weight within a batch. Surface variability is not specifically addressed in the Pharmacopoeia; however, this parameter can be monitored indirectly through a sufficiently large number of measurements. Batch uniformity must be assessed using the Ph. Eur. mass-uniformity test, in which one unit consists of 20 pilules. Twenty such units are selected at random, weighed separately, and used to determine both the individual masses and the mean mass. The batch complies with the test if no more than two individual masses deviate from the mean by more than 10%, and none deviates by more than 20%.

Our own assessment of the uniformity of a 20-pilule series yielded the following results: the lowest measured mass deviated by only 3% from the mean, whereas

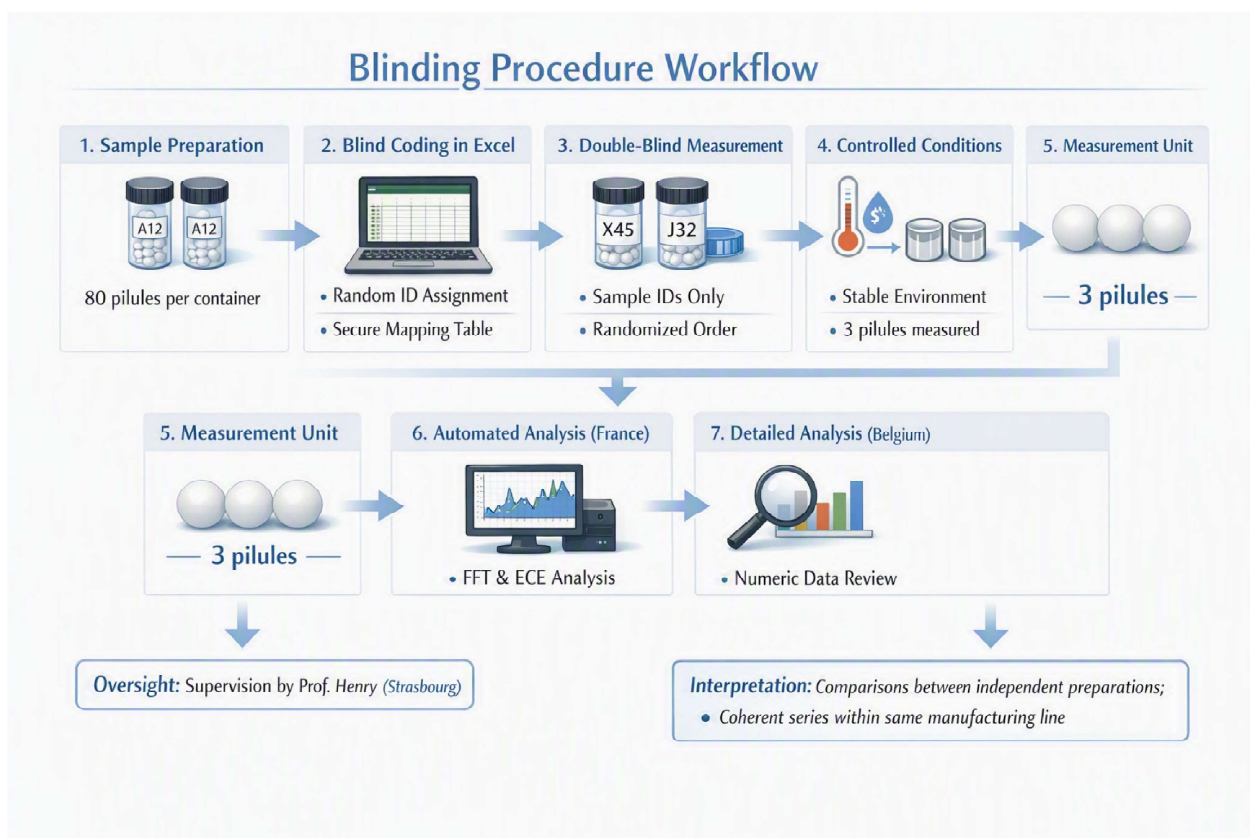
the highest was 4% above the mean, which is well within the limits specified by the *European Pharmacopoeia*.

The impregnation process was carried out using liquid preparations containing 70% *v/v* ethanol, at a ratio of 1 part by volume of liquid to 100 parts by mass of pilules. Impregnation was performed manually in Pyrex beakers that had previously been washed with deionised water and dried in an oven at 130 °C for 1 h 30 min.

Drying was performed under laminar-flow conditions at room temperature (21–25 °C), with the vessel rotated slowly and gently. Once completely dry, the granules moved freely within the vessel without adhering to its surface. Residual solvent content was not determined; however, the pilules were required to be fully dry before storage in closed, airtight tubes.

The *European Pharmacopoeia* does not require these measurements for a validated manufacturing process. Good pharmaceutical practice (GPP) requires that the manufacturer demonstrate control of the critical drying step. During process validation, the manufacturer must show that the procedure removes alcohol in a reproducible manner. Excessive residual moisture would lead to sticking or agglomeration of the pilules in the tube, and this is also addressed during validation of the manufacturing method. The technique used here corresponds to the validated procedure and does not need to be revalidated for each production batch. Moreover, all the preparations examined in this study, except for the oldest samples, were produced under controlled conditions in the same GPP-compliant laboratory. In our case, impregnation followed a standardised protocol, always the same. The premises are separate from the pharmaceutical area, and the temperature there is always between 20 and 25 °C to ensure the consistency of the masses of given volumes of ethanol solutions. A laminar-flow system ensures ISO 14644-2:2015 air quality throughout the laboratory. The relative humidity is recorded and is usually between 25 and 35%. Under these conditions, the drying time remains constant and is considered complete after 10 min. This timeframe is necessary to ensure that the pilules move freely and separately from one another in the drying bottle.

Blinding process: Blind numeration (blinding/coding) of samples in Excel (Microsoft Excel [365], Microsoft Corporation, Redmond, WA, USA) was achieved by assigning unique, random identifiers to samples, creating a mapping table, and securing it to prevent breaking the blind. A double-blind design was necessary, with randomised measurement order within each acquisition session, in order to minimise the influence of environmental variability, even though these conditions remained reasonably stable. The decision to record only three measurements from three pilules impregnated with the same preparation was likewise intended to preserve controlled external conditions. Under these circumstances, comparisons between two independent preparations should be regarded as indicative only. As noted above, however, the preparations within a given manufacturing line are not fully independent because the process is stepwise and the composition of each impregnating liquid depends on the preceding stage. The composition of these preparations is already characterised (NTA, EDX, and FTIR; see Discussion). They contain the same particulate and molecular components, differing mainly in their relative proportions, which are determined by the source material used at the start of the manufacturing process. Accordingly, the successive stages may reasonably be considered as a coherent series, allowing 81 measurements (3×27) to be included in the statistical analysis rather than only 3. The primary analysis of the photographs at the measurement laboratory in France was automated and was therefore performed blindly on site, including FFT processing and the primary energy–contrast–entropy analysis. MoreM detailed analyses were subsequently carried out in Belgium, but still on the basis of the sample numbering system and under the supervision of Prof. Henry in Strasbourg.



6. Conclusions

The present exploratory study suggests that size-6 pilules impregnated with potentised preparations may show measurable EPA- and FFT-derived image patterns under the experimental conditions used here. Within this dataset, some of these patterns appeared to vary according to source material (*Cuprum metallicum* versus *Gelsemium sempervirens*) and manufacturing protocol (Hahnemannian versus Korsakovian). However, these observations should be interpreted as preliminary image-analysis findings rather than as specific electrophotonic signatures or validated markers. Further measurements are required to determine whether such patterns are reproducible, whether they persist at high dilutions, and whether aged preparations can be reliably compared with freshly prepared samples.

Taken together, the findings support the rationale for further investigation of EPA as a complementary exploratory approach for describing image-derived differences in impregnated pilules. They do not establish that potentised preparations can be reliably differentiated from solvent controls or from one another on the basis of EPA alone. Larger, independently replicated studies, including predefined analytical criteria, external validation samples, improved environmental monitoring and complementary physicochemical measurements, will be necessary before any firm conclusion can be drawn about the analytical value of this method.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/chemosensors14090202/s1>. Statistical methodology—Academic report on MANOVA analysis—Access to a collection of original images.

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