i'Tec_H2
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Analysis method for microtracers

This analysis method was developed as part of research carried out into the homogenisation of feeds and was subsequently adapted to the issue of carry-over.
The method uses small magnetisable metal particles coated with a dye as a tracer. These two characteristics provide a source for measuring options. Magnetisation enables particles to be extracted from the mix.
Following extraction, the company marketing these tracers (Microtracer Inc.) counts the particles following their separation (demagnetisation) and discolouration on sheets of filter paper. This method does not permit high incorporation rates, in order to be able carry out particle counting.
It is because of this dependence on incorporation rates that Tecaliman has developed a colorimetric analysis method for microtracer concentration (µT). Numerous studies have enabled this to be validated. Its practical implementation has evolved over the years, but its general principle has not changed fundamentally.

1. Measuring principle

The principle of analysis is as follows (Figure 1):
Accurate weighing of the sample.
Magnetic extraction of the µT.
Transfer of the µT into a vessel.
Dissolving, Stirring and Filtration.
Reading the optical density using a wavelength that matches the µT dye.
Calculation of the corresponding quantity of µT by referring to a standard range.
Calculation of the mass concentration of µT in the mix by referring to the weight of the sample.

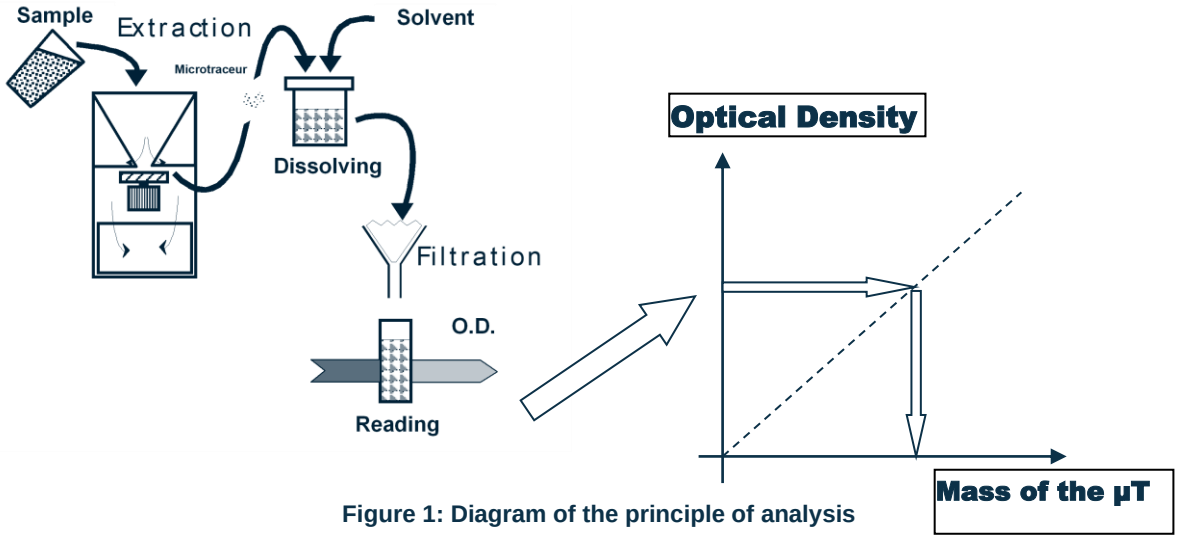

Figure 1: Diagram of the principle of analysis

2. Characteristics of microtracers

Different types of µT are marketed. The commercial names of these µTs primarily define the size of particles (Table 1). Secondly, they provide the colour of the dye carried by these particles. These dyes are always feed colourings.

Type of µT	Size
F	300 µm
FS	220 µm
RF	90 µm
RF superfine	30 µm

Table 1

Thus, the blue widely used by Tecaliman is Brilliant blue (E 133).

Finally, the lake information specifies that the dye is attached to the particles and that it can only be dissolved in an alcoholic and alkaline solvent. A lack of this information means that it is not attached and there will certainly be a loss of dye in the mix, making it difficult to link the mass of µT with the colour that develops following extraction. The µT used by Tecaliman is RF-blue lake, the physical characteristics of which appear in Table 2.

Median diameter (laser diffraction)	92 µm
Apparent (bulk) density	2.7 g/l
Tap density	2.9 g/l
Particulate matter density	6.6 g/l
Number of particles/g (Calculation)	$7.0\ 10^6$
Hausner ratio	1.06
Angle of repose	35.6°
Smallest flow diameter	< 4
Dust emissions (mg/25 g)	1.5

Table 2: Characteristics of the RF-blue lake µT

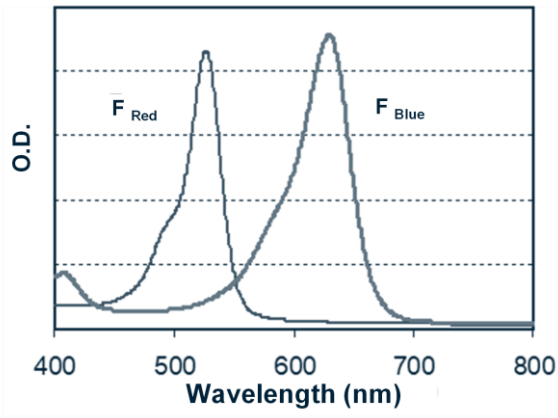

Figure 2: Absorption spectrum of red and blue µTs

The absorption spectra of dyes used for the red and blue µTs have peaks at the following wavelengths respectively: 525 nm and 629 nm (Figure 2). Should two tracers be combined and used in the same mix, it is possible to easily trace the blue µT, however, in this case, detection of the red tracer is slightly disrupted by the presence of the other one.

3. Analysis protocol

The protocol is the one applied by Tecaliman to measure the RF-blue lake µT during homogeneity and carry-over tests.

3.1. Incorporation levels and method

The level has been set at 250 ppm. The µT is mixed with rofelys at 12.5 % for introduction of the premixture at 2 kg/t in accordance with regulations.

3.2. Laboratory processing of samples

Samples are not ground or remixed as, in the case of single analyses of samples to study homogeneity, the test specimen equates to the entire mass of the sample. For duplicate analyses, samples are immediately divided using a riffle splitter.
For carry-over, samples are grouped in accordance with the established protocol (See **i'Tec_T2**).

3.3. Weighing and Extraction

The sample's mass is accurately weighed (0.1 g). For homogeneity tests, it is often between 250 and 300 g.
Extraction is carried out using a device designed by Microtracer Inc. equipped with a rotary magnet with a diameter of 70 mm, over which the entire mass of the sample is passed in the form of 1.5 cm thick bed.
Before the sample passes over the device, a thin plastic disc is placed on the surface of the magnet. This disc allows the µT collected by the attracting magnet to be extracted and transferred to a vessel. The extraction operation can be repeated several times (2 to 4) until the quantity of µT extracted has been depleted.

3.4. Dissolving and filtration

The solvent used in the case of the Lake microtracer has the following composition:

- 50 % ethanol
- 45 % water
- 5 % sodium hydroxide at 1 mol/L

50 ml of solvent is conventionally used, but a smaller volume can be used (up to 2 ml) to obtain an optical density that is within the range. To aid solubilisation of the dye, the µT/solvent mix is stirred for 45 seconds. A reading must be taken quickly, as the colour changes over time. Stabilisation of the latter can be obtained by neutralising the sodium hydroxide after solubilisation of the dye. A quantity of 0.2 ml of 25 % sulphuric acid is needed for 50 ml of solvent. The fraction of the solution subjected to optical reading is filtered to eliminate particles above 20 µm.

3.5. Optical Density (O.D.) reading

Reading the light absorbed at 629 nm enables the quantity of µT present in the sample and, therefore, the concentration to be deduced by applying the regression established between these two variables (Figure 3). Corrections can be made should a different volume of solvent be used than that used to establish the range.

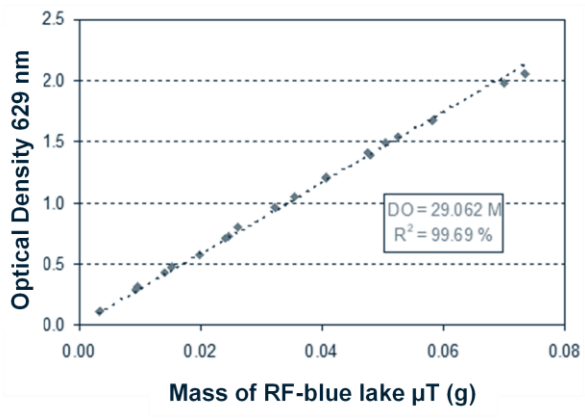

Figure 3: Example of the correlation between the O.D. and the mass of RF-blue lake µT (50 ml)

Other processes have demonstrated that the correlation broke down above an O.D. of 2.
Given the incorporation rates, the standard sample masses produce O.D.s of between 0.5 and 2.

4. Variation factors

4.1. Colorimetric range

A number of tests have highlighted the fact that the measurement used to determine the gradient of the relationship between optical density and the mass of µT could fluctuate slightly with a coefficient of variation of close to 3 %. This variation may be caused by various factors including:

- Weighing errors
- Errors in the volume of solvent
- Incomplete dissolving of the dye
- Poor filtration
- Errors in O.D. measurements

It should be mapped against that which occurs when conducting duplicate analyses on the same sample. Numerous tests carried out by Tecaliman have shown that the residual coefficient of variation that existed at the time was often also close to 3%.

4.2. Supplies

One of the most significant sources of variation remains the amount of colour attached to the metal particles, as shown by the curves plotted by way of an example, for two different deliveries (Figure 4). This variation results in the need to map a colorimetric range for each delivery of µT.

It has also been demonstrated that the quality of the ethanol has an effect on the correlation.

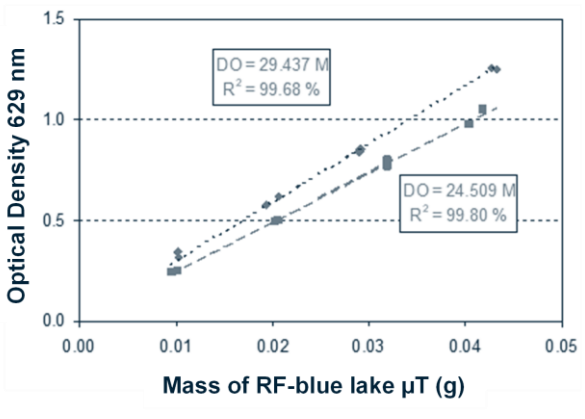

Figure 4: Example of correlations between the O.D. and the masses of 2 sources of RF-blue lake µT (weighed following extraction from the premix - 50 ml)

4.3. Extraction problems

This problem was, in part, highlighted by the following test: masses of µT were placed in pots and then mixed with 100 g of feed placed in each of the pots, and finally extracted (Figure 5).

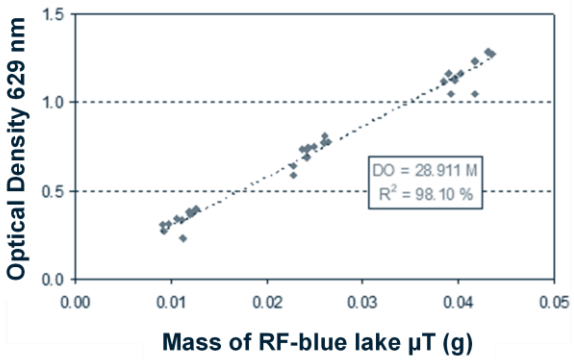

Figure 5: Correlation between the O.D. and the mass of RF-blue lake µT (weighing the µT – mixing with feed and extraction - 50 ml of solvent)

This simple test reveals optical densities that are below the expected values for several points that, therefore, equate to extraction errors.
In certain industrial tests, including for example those involved in the campaign to assess the homogeneity of feeds for laying hens at the plant outlet (Report no. 166. 2000), carried out with RF-blue lake (90 µm), three results had to be disregarded because of recovery rates of between 60 and 70 %.
Within the framework of tests on carry-over, a few lower recovery rates (50 %) were noted with the RF superfine red µT (30 µm), but the results were not disregarded as this loss of tracer could be explained by the contamination behaviour that was measured.

4.4. Presence of liquids

A study was carried out on three 100 kg mixes of a cattle feed (280 µm) containing 250 ppm of the RF-blue lake µT, to which water, soybean oil or molasses were added. After premixing for one minute, 1 % of the liquid is sprayed into the mixer, stirring continues for 5 minutes and then 10 samples are taken from the mixer. The operation is repeated 5 times in order to add approximately 1, 2, 3, 4 and 5 % of the liquids.
Microtracer analyses are carried out on 10 x 100 g samples without repetitions.
Logically, the tracer recovery rate proved to be sensitive to the quantity of liquid added for the three liquids (Figure 6).

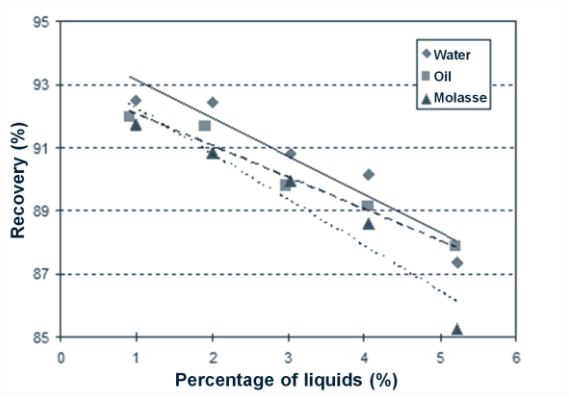

Figure 6: Percentage of tracer recovered depending on the incorporation rate for liquids

Without liquids, the recovery rate is close to 94 %, which is the value found if the lines are continued for zero incorporation of liquids. If the liquids are compared, it should be noted that the rates are always lower with the most viscous products, such as molasses, followed by oil with water in a better position. For water and molasses, the recovery rate appears to experience a more marked decrease when the percentage of liquid is increased from 4 to 5 %. Nevertheless, the lowest recovery rates are close to 85 % and cannot be considered as very poor. In effect, they are in the range of what can be obtained with certain additives without the addition of liquids to mixes.
Moreover, the coefficient of variation was not modified by this incorporation of liquid.
It can be concluded from recent results relating to carry-over that this sensitivity to the presence of liquids could be more significant in an industrial setting or with a finer µT.

5. Conclusions

This method developed almost 10 years ago by Tecaliman has advantages and drawbacks:

Drawbacks

A magnetic device must not be installed on an industrial site between the place where the tracer is incorporated and the sampling location.
It is not suitable for tests on pellets or feeds with a high liquid content.
There is a variation in the quantity of dye depending on the batch of µT
There are extraction problems

Advantages

- The low cost of analysis (around 100 F), which makes it competitive with that for trace elements.
- The µT is inert and compatible with the consumption of feeds.
- With use of the Lake type, there is no loss of dye even in the presence of free water in the feed (passing through the press), which prevents colouring of the latter.
- The µT is an external tracer, which is never present in raw materials. Consequently, it is not present in plants prior to its introduction for testing, which limits the risks of results being distorted by possible random contamination or significant “background noise”.
- Analysis relates to the entire mass of samples, which limits the risks of analytical error that exists when switching from the size of the sample collected in the plant to that of the test specimen.
- Possible adaptation (which is actually hard to implement in practice) of the size of the sample in order to match the animal's daily feed intake may be undertaken by varying the incorporation rates or the quantity of solvent.
- This adaptation is also used in the case of carry-over, by enabling the size of the sample and the quantity of solvent to be adjusted when searching for traces with a degree of reliability.

6. Bibliography

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Internal Report no. 166. Jan. 2000. Essais industriels d’évaluation de l’homogénéité d’aliments pondeuses en farine à la sortie de 16 sites industriels.