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FERROMAGNETIC NANOPARTICLES OF IRON/COBALT AND IRON/MANGANESE OXIDES IN AQUEOUS SOLUTIONS OF SURFACTANTS

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Nanoparticles of iron/cobalt and iron/manganese oxides in the form of stable suspensions are used in the development and manufacture of nanotracers, which are used to check the mixing processes of animal feeds, which are in both solid and liquid states, the coding of liquid additives and the efficiency of their uniform distribution in prepared premixes and compound feed [1,2].

1. Sakhno T., Panchenko V., Semenov A., Barashkov N., Kharak R., Sakhno, Y. Ukrainian Metrological Journal. 3, 55 (2024). DOI: 10.24027/2306-7039.3.2024.312481.
2. Sakhno T., Panchenko V., Semenov A., Barashkov N., Sakhno, Y. Ukrainian Metrological Journal. 2, 54 (2025).

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Suspensions of nanotracer nanoparticles must be stable, and feed mixtures must be homogeneous. Since feeds are multicomponent mixtures, a statistical approach is used to assess the distribution of particles in the mixture and, thus, determine the quality of their mixing [3,4].

3. Sakhno T., Semenov A., Barashkov N. Grain Products and Mixed Fodder’s, **20** (2, 78), 32 (2020).

4. GMP+. Currently this is Technical Specification TS1.11 – Control of residues and uniformity. EN version: January 1, (2022).

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Nanotracers, which are used as markers for the analysis of feed mixing, contain information about the type and specific feature of agricultural products and its genetic composition [5].

5. Daumann, B., Nirschl, H. Powder Technology, **182**(3), 415 (2008).

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- This work presents methods for obtaining ferromagnetic nanotracers based on iron/cobalt and iron/manganese oxides and studying their ability to form stable solutions of aqueous suspensions in the presence of surfactants.

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The work presents step-by-step methods for obtaining ferrofluids based on iron/cobalt and iron/manganese oxides and their subsequent dispersion in aqueous solutions of surfactants.

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The annual production capacity of the global compound feed industry is over 500 million tonnes, indicating the significant size and importance of this sector in agriculture . Producers waste labour, energy and capital when they mix feeds for longer than necessary to obtain a homogeneous mixture. This excessive mixing can lead to the decomposition of vitamins and drugs or cause segregation. But if the feed is not mixed completely, portions may contain either too much or too little of the required ingredients. This excessive variability can lead to economic losses for feed users and can increase the amount of pharmaceutical residues. Periodic routine testing of the mixer is justified from an economic and ethical point of view. In the mid-1990s, regulatory authorities in many countries paid special attention to ensuring that medicated feeds were mixed thoroughly, with the aim of ensuring that all micronutrients were added in accordance with the requirements expected of the feed.

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Homogeneous mixing of ingredients is critical to ensure that feeds meet the nutritional needs of different livestock and poultry species. Achieving homogeneity in the feed formulation ensures even distribution of nutrients and minimizes the risk of segregation of components, which can lead to imbalances in animal nutrition [6]. To solve these problems, modern mixing technologies are used, such as ribbon mixers and vertical blenders, which are equipped with intelligent control systems. These systems allow for rapid adjustments to the feed formulation based on real-time data, which increases the efficiency of the production process.

[6] Sakhno T., Semenov A., Barashkov N. Grain Products and Mixed Fodder's, **20** (2/78), 32 (2020) doi: https://doi.org/10.15673/gpmf.v20i2.1763

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Cobalt and its biological role

It is known that the activity of various vitamins is activated by trace elements, such as vitamin B12, which includes cobalt. Trace elements enhance the activity and synthesis of some vitamins. Cobalt affects the body through vitamins, enzymes and hormones.

Cobalt, together with other trace elements, increases the activity of vitamins, hormones and enzymes and activates the metabolism, especially of minerals, as well as carbohydrates and proteins. Cobalt also contributes to better absorption of nutrients in feed and better hematopoiesis. Cobalt, as well as a number of other trace elements, improve protein synthesis, which contributes to the development and growth of animals, increased respiration and improved productivity and reproductive ability of animals.

Lack of cobalt in feed is a consequence of a decrease in the synthesis of vitamin B12. Cobalt synthesis in ruminants occurs mainly in the rumen. With a lack of cobalt in the animal body, their ability to reproduce may be impaired. If cows have a cobalt deficiency, they often give birth to weak calves with low resistance and growth retardation.

Cobalt and its biological role

After increasing the cobalt content in the diet of such cows, they have an increase in blood volume and an increase in the content of vitamins in the body, which results in an increase in their body's resistance to diseases and an improvement in their fertility.

Cobalt and vitamin B12 are hematopoietic substances, they also improve protein synthesis in the animal body, which leads to an increase in live weight in animals, an increase in milk yield and growth of young animals.

Cobalt (like iodine and zinc) enhances the activity of the reproductive organs in animals, improves the fertility of animals, and the offspring of such animals develop and grow better.

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Manganese and its biological role

Manganese deficiency can lead to serious health problems in animals, such as impaired bone formation (perosis in birds), reduced reproductive capacity and growth retardation. In such cases, compound feed with manganese supplements is an effective way to restore normal functioning of the body.

Thus, manganese is an important element in compound feeds, necessary for maintaining health, growth and productivity of animals. Its correct dosage and use help to ensure the proper level of this trace element in the diet of animals, which has a positive effect on their development and production indicators.

It is also involved in many biological processes, including the formation of connective tissues, bones, blood clotting factors, sex hormones and in antioxidant protection. Mn deficiency causes hypercholesterolemia, impaired glucose tolerance, infertility and skeletal abnormalities [9]. Erdogan et al. [10] reported that the RDA of Mn for an adult is 2.3 mg/day, while a study [19] indicated that the Mn content was 10.7 ± 0.00 mg/kg, for compound feeds the Mn content did not exceed 500 mg/kg, i.e. the upper limit of Mn defined by WHO/FAO [11].

[9] F.S. Al-Fartusie, S.N. Mohssan, Indian J. Adv. Chem. Sci**.** **5** (3) (2017) 127–136, https://doi.org/ 10.22607/IJACS.2017.503003

[10] M.E.S¸ Erdogan, ˘ S.D. Kahraman, Biol. Trace Elem. Res. **201** (3), 1488 (2023), https://doi.org/10.1007/s12011-022-03217-3.

[11] H. Can, I.I. Ozyigit, M. Can, A. Hocaoglu-Ozyigit, I.E. Yalcin, Biol. Trace Elem. Res. **199**, 1123 (2021). https://doi.org/ 10.1007/s12011-020-02208-6.

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Manganese and its biological role

An increase in manganese in supplements can have negative consequences, so it is important to adhere to the recommended norms. Excessive consumption leads to toxic effects: disruption of the nervous system, damage to internal organs, loss of coordination, decreased appetite and general deterioration of health. In addition, excess manganese interferes with the absorption of iron, zinc and copper, which causes their deficiency and worsens the condition of animals. This reduces productivity: slows down weight gain, worsens the quality of meat and milk, reduces egg production in poultry. Also, excessive use of manganese supplements increases feed costs without a real improvement in productivity, which is economically unprofitable. Therefore, controlling the level of manganese in compound feeds is critically important for animal health and efficient production [12].

[12] Research-Based Information That You Can Use G2340 · Index: Animal Agriculture, Beef Issued February 2022 Formulation Considerations for Mineral and Vitamin Supplements for Beef Cows Mary Drewnoski, Beef Systems Specialist, University of Nebraska–Lincol

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Ferromagnetic microtracers

In feed production, it is important to create a completely homogeneous mixture. In multi-component compound feeds, a statistical approach is used to assess particle distribution and quality [13]. Using this method makes it possible to understand whether the ingredients are well distributed in the mixture or, in other words, whether each portion of the mixture is homogeneous. Currently, microtracers are used to check the homogeneity of feeds according to the TS1.11 standard - Control of residues and uniformity [14].

[13] Eisenberg S. U.S. Patent No 4,152,271, San Francisco: Tracer-containing composition. 1974-01-28 Application filed by Micro Tracers Inc.; 1979-05-01 Publication of US4152271A. United States Patent. Available at: https://patents.google.com/patent/US4152271

[14] GMP+. Currently this is Technical Specification TS1.11 - Control of residues and uniformity. EN version: January 1, 2022. Available at: https://www.gmpplus.org/media/52ipu4f3/ts111-control-of-residues-homogeneity-en.pdf

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Ferromagnetic microtracers

The American company MicroTracers Inc., located in San Francisco, USA, patented and manufactures ferromagnetic microtracers (MT) [15, 16]. According to the method proposed by the company, 50 g of ferromagnetic microtracer (MT) for each ton of compound feed is introduced into the mixing equipment as a microadditive during feed preparation. The company offers three types of microtracers, which have adsorbed food dyes on their surface registered by the Food Drug Administration, USA.

- One type of microtracer (Microtracer F) contains iron particles,
- the second type of microtracer (Microtracer FS) contains stainless steel particles with a size of 150-350 μm,
- and the third type of MT (Microtracer RF) contains particles of reduced iron powder with a particle size of < 150 μm.

[15] Eisenberg S. Microingredient containing tracer. U.S. Patent No 4,654,165. 1985-04-16 Application filed by Micro Tracers Inc., 1987-03-31 Publication. United States Patent. Available at: https://www.freepatentsonline.com/4654165.pdf

[16] Mendigaliyeva S.S., Irgibaeva I.S., Barashkov N.N., Sakhno T.V., Aldongarov A.A.. Reports of the National Academy of Sciences of the Republic of Kazakhstan. **345**(1), 282 (2023). doi: https://doi.org/10.32014/2023.2518-1483.201

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Ferromagnetic microtracers

When formulating vitamin, mineral or therapeutic premixes, the microtracer serves to indicate the presence of the premix in finished feeds, as well as to identify feed additives and feeds containing such additives that are patented. In quantitative analysis, MicrotracersTM can be used both to document mixing efficiency and the adequacy of "cleaning" of mixers and other feed production equipment from batch to batch.

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Ferromagnetic nanotracers

Iron-based microtracers are not suitable for mixing liquid feeds, as well as coding liquid additives (enzymes), and for determining their distribution in premixes and finished feeds. Therefore, a magnetic retractable **nanotracer** with particles based on iron oxide, which is a magnetic liquid, was proposed for liquid feeds [16]. The nanotracer has **nanometer-sized particles** and high dispersion, due to which it is evenly distributed in a liquid medium.

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Research and its methodology

According to standard requirements for compound feed, mixers must be characterized by effective mixing.

Mixing capacity	**Coefficient of variation**
1:10000	≤ 15%
1:100000	≤ 10%

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Research and its methodology

According to the standard [17] when using direct methods, the probability (p%) is defined as:

Probability (p%)	**Homogeneity**
≤ 1%	insufficient; likely significant deviation;
1% < p < 5%	likely significant deviation; cannot be stated unambiguously. The test must be repeated;
p ≥ 5%	good homogeneity

The results of the studies were statistically confirmed to determine the coefficient of variation, which is a value for assessing the quality of the mixing process according to these criteria

[17] GMP+. Currently this is Technical Specification TS1.11 - Control of residues and uniformity. EN version: January 1, 2022. Available at: https://www.gmpplus.org/media/52ipu4f3/ts111-control-of-residues-homogeneity-en.pdf

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Research and its methodology

The number of particles was determined by counting colored dots on filter paper by eye or using the computer-aided TraCo image assessment and analysis tool [18]. To obtain accurate results, statistical estimation should be performed using the Poisson distribution.

[18] GMP+ International. S9.14 Examples of methods for measuring carry-over and homogeneity. Version: March 1, 2021. Available at: https://www.gmpplus.org/feed-certification-scheme/scheme-documents/support/s914#h1

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The process of obtaining a magnetic fluid

Main stages:

(I) Preparation of a ferromagnetic fluid (ferrofluid). [19];

(II) Ensuring the stabilization of a ferromagnetic microtracer in an aqueous medium by dispersing drops of iron fluid using surfactants [20].

[19] R.D. Crapnell, C.E. Banks, Sensors Actuators Rep. **4** (2022) 100110, https://doi.org/10.1016/j. snr.2022.100110.

[20] F.S. Al-Fartusie, S.N. Mohssan, Indian J. Adv. Chem. Sci. **5** (3) (2017) 127–136, https://doi.org/ 10.22607/IJACS.2017.503003

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(I) Preparation of ferrofluid based on iron and manganese oxides [1,2,21]

(1) Prepare an aqueous solution containing 0.95 M $FeCl_3 \cdot 6H_2O$, 0.44 M $FeCl_2 \cdot 4H_2O$ and 0.11 M $MnCl_2 \cdot 4H_2O$ (or 0.44 M $FeCl_2 \cdot 4H_2O$, 0.95 M $FeCl_3 \cdot 6H_2O$ and 0.09 M $CoCl_2 \cdot 6H_2O$) and mix with a non-magnetic rod;

(2) separately add 350 ml of water to 350 milliliters of 28% aqueous ammonia solution;

(3) add the ammonia solution to the iron salt solution within 75 seconds;

(4) heat the colloidal magnetite to 90^{o}C with constant stirring and heating;

(5) add 40 ml of oleic acid to 460 ml of heptane and heat to 90^{o}C;

(6) mix solutions (4) and (5) when their temperature exceeds 90°C;

(7) stir solution (6) for 15 minutes;

(8) remove the upper organic layer.

The resulting liquid is a stable ferromagnetic liquid with physical characteristics:

Density - 1.038 g/ml;

saturation magnetization - 252 Gauss;

viscosity - 4.3 cP.

[21] Dogan N., Dogan O.M., Irfan M., Ozel A.S., Kamzin F., Semenov A.S., Buryanenko I.V. Journal of Magnetism and Magnetic Materials, **561**, 169654 (2022) doi: https://doi.org/10.1016/j.jmmm.2022.169654

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(II) Dispersion of ferrofluid in 0.5% aqueous surfactant solution [1,2,21]

Ammonium oleate or dimethylamine salt of oleic acid (DMAOC) were used as surfactants.

(1) Ferrofluid was dispersed in 0.5% aqueous surfactant solution with a mass ratio of ferrofluid to surfactant solution from 1:100 to 1:400;

(2) ferrofluid mixed with surfactant was filtered through Whatman filter paper with a pore size of 5 microns;

(3) the size of nanoparticles of stable suspension was determined

(4) the manganese/cobalt content in nanoparticles was determined by colorimetric method.

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Testing $Fe_xMn_yO_z$ and $Fe_xCo_yO_z$

Ready-made stable suspensions of nanoparticles $Fe_xMn_yO_z$ or $Fe_xCo_yO_z$, which contain more than 95% iron and less than 5% Mn or Co, respectively, are used for coding liquid feed additives (enzymes), for checking the distribution of liquid additives in compound feeds and premixes, and for controlling the quality of feed mixing.

The prepared suspensions of nanoparticles $Fe_xMn_yO_z$ and $Fe_xCo_yO_z$ were tested in laboratory and production conditions to assess the quality of liquid feed mixing and coding of liquid additives.

In laboratory experiments, a 100 ppm nanotracer suspension was mixed with the enzyme in a liquid state (enzyme concentration in the feed 110 ppm) and added to the feed.

After that, the ferromagnetic nanoparticles were extracted.

$Fe_xMn_yO_z$ and $Fe_xCo_yO_z$ nanoparticles were used as magnetic markers.

In laboratory tests, at least 75% recovery was observed [1,2].

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Testing $Fe_xMn_yO_z$ and $Fe_xCo_yO_z$

Industrial trials of liquid nanotracers were conducted with a large European feed manufacturer. To test the mixing quality of liquid ingredients in industrial trials, 0.2% of a stable nanotracer suspension was added to the ingredients of 500 kg of pig feed mixture. Interpretation of test data was performed using Poisson and χ^2 statistics. By analyzing a series of optical density values as a Poisson distribution, the homogeneity of the prepared mixtures was assessed. The results obtained included both complete mixing (when the probability was > 5%), intermediate (when the probability was > 5%) and incomplete mixing (when the probability was < 1%).

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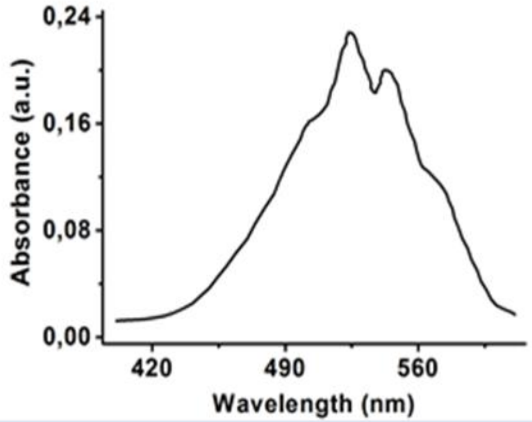
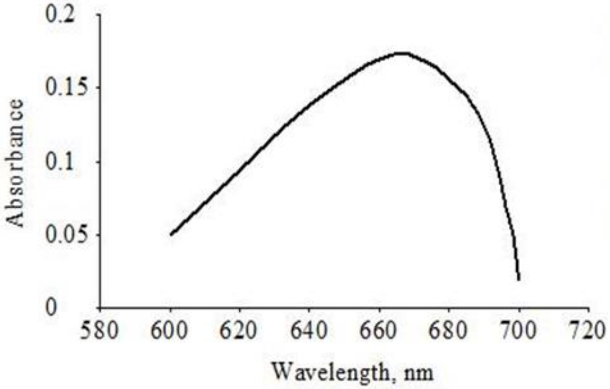
Determination of manganese/cobalt concentrations by modified spectrophotometric analysis of complexes



Manganese-containing ferromagnetic nanoparticles $Fe_xMn_yO_z$ obtained from a suspension using a plastic-coated neodymium magnet were dissolved in 20% aqueous HCl and oxidized to potassium permanganate ion with periodate. Spectrophotometric study of the optical density of permanganate anions was carried out at a wavelength of 525 nm, because it was at this wavelength that the highest and most pronounced absorption coefficient was (3220 $M^{-1}\cdot cm^{-1}$) (Fig. 1) [2].



The $Fe_xCo_yO_z$ emulsion was extracted with hydrochloric acid to give $CoCl_2$, which in the presence of NH_4SCN, ammonium acetate, and diethylamine formed a blue complex. Spectrophotometric study of the cobalt thiocyanate-diethylamine complex in dimethyl sulfoxide has an absorption maximum at a wavelength of 667 nm (Fig. 2) [1].

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Nanoparticle Tracking Analysis (NTA)

The manganese and cobalt content in the obtained stable aqueous suspensions was determined by the colorimetric method. To determine the sizes of nanotracers, an innovative statistical approach NTA (Nanoparticle Tracking Analysis) or nanoparticle trajectory analysis was used, which allows studying and visualizing the size and concentration of nanoparticles in a solution by tracking the Brownian motion of an individual nanoparticle using a microscope in its field of view

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[22] Foreman-Ortiz I.U., Ma T.F., Hoover B.M., Wu M., Murphy C.J., Murphy R.M., Pedersen J.A. *Colloid Interface Sci.* **615**, 50 (2022)

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Nanoparticle Tracking Analysis (NTA)

For dispersion in solutions, surfactants of different nature were used, namely: dimethylamine salt of oleic acid (DMAOA) and ammonium oleate. NTA showed the distribution of particles by their size and made it possible to conclude about the effectiveness of each of the surfactants in the processes of stabilizing nanoparticles and preventing aggregation. It was found that for dimethylamine salt of oleic acid (DMAOA) the average particle size is 90-120 nm, and for ammonium oleate - 100-130 nm. Ammonium ammonium oleate demonstrates a wider particle size distribution compared to dimethylamine salt of oleic acid. In the ammonium oleate solution, nanoparticles have good stability, but less than in the DMAOA solution. Ammonium oleate, despite the effectiveness of ammonium oleate, a wider particle size distribution is observed in it.

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CONCLUSIONS

- **A method for obtaining ferromagnetic nanotracers $Fe_xMn_yO_z$ and $Fe_xCo_yO_z$ based on iron and manganese oxides and iron and cobalt oxides has been developed, which makes it possible to obtain stable suspensions of nanotracers in aqueous solutions of surfactants.**

- **The particle size of nanoindicators was determined using the Nanoparticle Tracking Analysis (NTA) method, based on direct visualization and analysis of nanoparticles in aqueous solutions of two types of surfactants.**

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CONCLUSIONS

- **The possibility of using nanotracers $Fe_xMn_yO_z$ and $Fe_xCo_yO_z$ to assess the quality of mixing liquid feeds in laboratory conditions was demonstrated.**

- **It has been shown that the surfactant dimethylamine salt of oleic acid (DMAOA) is best suited for dispersing ferromagnetic nanoparticles in suspension to determine the quality of compound feed mixing, as it creates a more uniform particle size distribution and is more stable, which is important for reliable and accurate assessment of the quality of the feed mixture.**

THANK YOU FOR YOUR ATTENTION!