Research Article

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The use of UV-induced fluorescence for the assessment of homogeneity of granular mixtures

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Abstract: This paper presents the results of fluorescence-based analysis of homogeneity of five multicomponent granular mixtures. Analyses were performed using solutions of selected substances capable of emitting light following UV irradiation, namely Tinopal 0.03% and Rhodamine B 0.01%. Mixtures were spiked with the key component consisting of maize grains coated with the fluorescent solution. The tracer content was determined on the basis of computer image analysis, and the results were compared to those obtained using the traditional weighing method. On this basis, the proposed method was verified and assessed for applicability in estimating the homogeneity of mixtures comprised of 8, 10, 11, 14, and 20 components. The results suggest that both Tinopal and Rhodamine B may be used to estimate the tracer content in the tested mixtures.

Keywords: fluorescence; homogeneity; granular mixture; tracer.

1 Introduction

The mixing of granular materials is a key process in numerous branches of industry, including the agri-food industry where an increase in animal feed production has been observed for several years. Appropriate homogeneity of the feed mixtures determines their energy and nutritional value, while simultaneously warranting an appropriate use of these mixtures; therefore, homogeneity is an important quality indicator [1-4]. Mixing is a key process in feed production. The importance of mixing as well as factors affecting the correctness of the process have been described by many authors [3, 5-16]. Many years

of research were insufficient to exhaust the issue, thus warranting further studies being conducted and novel solutions being employed for that purpose.

Following appropriate mixing time, random mixing status is attained. This means that the likelihood of finding a particle of a particular component is the same at every point within the bulk of the mixture. In the case of systems consisting of multiple different components, characterization of mixing status and homogeneity is one of the key analytical problems [5, 17]. Incomplete homogeneity can be achieved when mixing this type of system, and the status of the entire mixture may only be estimated on the basis of collected samples [18]. In laboratory practice, analysts have at their disposal appropriate tools to observe the behavior of granular particles [19-22]. In industry, however, difficulties are still encountered in this regard. Solutions currently used in industrial practice usually consist of monitoring mixing status by determining the content of a key component in collected material samples [17, 23-25].

The use of fluorescence for detecting or separating selected material elements or tracking molecules has been the subject of many authors' research. In particular, the development of non-invasive methods based on fluorescence and their application to examination of the constituent materials of works of art were presented by Romani, et al. [26]. Fluorescence has also been used, inter alia, for: chase analysis of amino acid systems [27], detection of cysteine in bioliquids [28], biodegradable hydrogel using Rodamine B [29] or identification of chemical compounds [30].

In the novel method proposed by the author of this study, the key component in an agri-food mixture consists of a selected mixture component (in this case, maize grains) coated with a fluorescent substance. The coated ingredient is introduced to the mixer because it is detectable via fluorescence following ultraviolet radiation. As a result, the quantity of the key component in a sample may be determined accurately. The applicability of this approach has been confirmed by studies hitherto conducted by the authors. In the present study, the testing methodology is being presented, and an attempt was made

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Table 1: Composition of study mixtures.

Number of components	8	10	11	14	20	Density (kg/m ³)	Average size (mm)
Component	Share of component (%)						
Barley	-	19	-	-	-	600	3.42
Dari	1	10	4	13	9	725	8.14
Yellow maize	32	30	29	12	10	742	7.20
Popcorn maize, small	-	-	-	-	7	765	6.42
Red maize	-	3	6	7	6	714	3.85
Wheat	38	9	19	6	10	798	6.91
Pea mix	-	10	8	-	9	784	7.22
Green pea	4	-	-	-	-	780	7.81
Yellow pea	9,5	-	-	-	-	792	4.29
Black pea, small	-	-	3	-	4	790	4.54
Green pea, small	-	-	2	-	4	700	4.05
Sorghum	9	9	22	-	10	430	4.95
Sunflower	1,5	5	2	13	1	730	2.24
Millet	5	4	-	-	-	721	2.24
Red millet	-	-	3	3	2	732	2.26
Yellow millet	-	-	2	-	3	653	2.50
Rape	-	1	-	2	-	500	4.64
Safflower	-	-	-	14	11	550	3.48
Hemp	-	-	-	12	2	665	2.92
Hulled oat	-	-	-	5	-	800	5.57
Soybeans	-	-	-	5	-	700	2.28
Canary seed	-	-	-	3	1.25	685	2.15
Linseed	-	-	-	3	1.25	700	4.56
Mung bean	-	-	-	2	-	800	4.63
Vetch	-	-	-	-	3	550	3.23
Buckwheat	-	-	-	-	2.5	760	2.25
Hulled rice	-	-	-	-	2.5	600	2.34
Unhulled rice	-	-	-	-	1.5	615	3.28
Mean feed grain size (mm)	5.68	5.36	5.49	4.58	4.88	-	-

to determine whether the mixture composition might present limitations to the applicability of the method.

2 Methods

The study was carried out using five different multicomponent granular mixtures (Table 1, Figure

1). Each of the mixtures contained maize as one of the components.

A pour-through mixing station was used for the purposes of the study. A laboratory funnel flow mixer consisted of two identical containers with height of the cylindrical part of 200 mm and an inner diameter of 150 mm. Before each mixing, the mixing container was charged in the same manner to include 900g (90%) of feed

Figure 1: Feed mixtures used in researches.

and 100g (10%) of tracer. The key component, i.e. yellow maize, was wet-treated with tinopal and rhodamine B solutions. Tinopal was used at a concentration of 0.03% while rhodamine B was used at a concentration of 0.01%; both concentrations were determined to be optimal per the results of previous studies [25, 31, 32]. The properties of substances used are presented in Table 2.

After completion of the mixing procedure (10 flows), ten samples ($N=10$) of 40 g were collected from the total volume of the mixing bed. This part of the test was carried out in a workstation consisting of an UV-lit chamber, digital camera, computer system with Patan® image analysis software, and analytical balance with an accuracy of 0.01g. A sample of the mixture was placed in a Petri dish (120 mm x 20 mm). The collected samples were placed in a dedicated chamber to ensure air tightness and separation from any external factors. The chamber was equipped with UV lighting (two UV rays 2x15W) controlled from the outside. The sample was placed horizontally on a movable shelf, and then the chamber was closed. Irradiation of samples triggered the fluorescence of the tracer. A camera lens was placed centrally over the sample, and it was used to obtain photos in BMP format (1600x1200 pixels). The obtained images were subjected to computer analysis using the RGB-256 color scale with Patan® software by Marek Krótkiewicz. The application allows the selection of a specific component from the surrounding background.

In the first stage, three classes were segmented: 1- key component, 2 and 3 - background. In this process, each pixel of the image is assigned to the correct class in the RGB value. In the second stage, the tested surface was measured [33]. The obtained results of the key component content were verified against data obtained using a conventional method of manual separation and used to calculate the coefficient of variation as a parameter to assess the homogeneity of the mixture. The maximum acceptable difference between the results obtained by both methods was established at 5%. For each mixture, tests were carried out in triplicate.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

The evaluation and characterization of the mixing process of granular components is very complicated, due to a number of factors, including the variety of parameters that characterize mixing. In industrial practice, we are dealing with the mixing of many components that are characterized by a significant differentiation in terms of physicochemical parameters. Access to research and analytical methods applicable in such conditions is still limited. The proposed method using the phenomenon of

Table 2: Applied fluorescent substances and their concentration.

Fluorescent substances	Excitation (nm)	Emmision (nm)	CAS Registry Numbe	Molecular weight	Formula	Solution (%)
Tinopal	350	430	27344-41-8	562.6	$C_{28}H_{20}Na_2O_6S_2$	0.03
Rhodamine B	553	627	88-81-9	479.02	$C_{28}H_{31}ClN_2O_3$	0.01

Table 3: Percentage content of the key component and the results of Student's t-tests at $\alpha=0.05$.

Number of mixture	Share of key component ^a (%)		Difference (%)	t	p
	Method 1 ^b	Method 2 ^c			
Tinopal					
1	7.50±0.95	7.50±0.93	2.55±0.92	-0.004	0.99645
2	7.18±1.40	7.16±1.47	2.81±1.45	0.025	0.98020
3	6.75±1.24	6.70±1.28	2.22±1.55	0.084	0.93401
4	6.97±1.10	6.96±1.04	2.94±1.33	0.002	0.99844
5	6.39±0.87	6.32±0.90	2.95±1.35	0.168	0.86859
Rhodamine B					
1	7.15±0.86	7.13±0.90	1.54±0.69	0.048	0.96219
2	7.33±1.29	7.32±1.23	1.43±0.81	0.010	0.99206
3	7.17±1.24	7.12±1.19	2.12±0.83	0.076	0.93948
4	7.21±1.31	7.17±1.37	2.31±2.34	0.054	0.95749
5	7.21±1.02	7.10±1.05	2.73±1.37	0.230	0.82053

^a Mean of three analyses and then samples ± SD (standard deviation)^b Results obtained by the method using of computer image analysis^c Results obtained by traditional method, manual separation

UV-induced fluorescence to follow the key component in multicomponent granular mixtures seems to be a desirable and at the same time innovative tool. The results of tests of the method are presented in Tables 2 and 3, and a graphical interpretation was made (Figure 2).

Table 3 presents the results of the percentage content of the key component as obtained by both methods along with the results of the Student's t-test. Analyzing the data in Table 3, it can be observed that the results obtained using the fluorescence method are not significantly different from those obtained using the reference method. No cases of a difference exceeding the predefined threshold of 5% were observed. Differences obtained when Rhodamine B solution was used as the fluorescent agent were slightly lower compared to these obtained using Tinopal. However, the differences between both solutions were not significant (Figure 2). In addition, comparative analysis (Student's test for $\alpha=0.05$) revealed no difference in the tracer content results obtained using both methods.

Figure 2 provide a graphical interpretation of the differences between the results obtained by the two methods (i.e. computer image analysis and manual separation). The results confirm the conclusions presented above. Differences were lower when Rhodamine B solution was used as the fluorescent agent, particularly in the case of mixtures 1 and 3, which were mixtures comprised of 8, 10, and 11 components. No such trends were observed for Tinopal. The importance of this differentiation was analyzed by means of statistical analytical methods. The analysis consisted of demonstrating the significance of differences between the results obtained for five different mixtures ($r=5$). To this end, analysis of variance (ANOVA) was used at $\alpha=0.05$. The obtained results revealed a lack of statistically significant differences between the results obtained for five mixtures using Tinopal ($F=0.477$, $p=0.75218$) and Rhodamine B ($F=1.429$, $p=0.23991$). Thus, one may assume that the number of mixture components and the mean size of grains are not important limiting

Figure 2: Graph box plot of the difference in percentage share of tracer covered with Tinopal and Rhodamine B for the tested feed mixtures.

Table 4: Coefficients of variation (CV) of homogeneity of mixtures.

Number of mixture	Coefficient of variation CV (%)		Difference (%)
	Method 1 ^a	Method 2 ^b	
	Tinopal		
1	12.66	12.42	1.85
2	19.50	20.48	4.80
3	18.44	19.15	3.71
4	15.74	14.90	5.64
5	13.58	14.28	4.96
	Rhodamine B		
1	12.06	12.67	4.82
2	17.59	16.84	4.45
3	17.26	16.68	3.48
4	18.13	19.11	5.13
5	14.11	14.75	4.34

^a Results obtained by the method using of computer image analysis
^b Results obtained by traditional method, manual separation

factors for the use of fluorescence in the analysis of the key component content.
Analyzing the results presented in Table 4, it can be observed that in two cases, the results of feed

homogeneity exceeded the predefined threshold of 5%. This was the case for the mixture consisting of 14 components (mixture 4) with either Tinopal or Rhodamine B traced seeds. The magnitude of the differences were 5.64% for Tinopal and 5.13% for Rhodamine B. Mixture 4 was characterized by the lowest mean size of particles ($d=4.58$ mm). In the remaining cases, the predefined threshold was not exceeded. The lowest difference level of 1.85% was observed for mixture 1 for Tinopal only. The mixture consisted of the lowest number of components (8 components) and had the largest mean component size of 5.68mm. On the basis of the calculated differences between results obtained using both methods (Tables 3 and 4), one may conclude that the use of data pertaining to the content of a fluorescent-coated component for determination of feed homogeneity may be subject to error. Therefore, an analysis was performed to determine any significant difference between feed homogeneity results obtained using both methods. Results of a Student's t-test at the significance level $\alpha=0.05$ revealed no significant difference in the homogeneity of mixtures as determined using both methods for Tinopal ($t=-0.129$, $p=0.90021$) as well as Rhodamine B ($t=-0.113$, $p=0.91311$). The analysis of the results did not demonstrate any effects of the key parameters, such as the number of components and the mean grain size, on the applicability of the fluorescence

method in the assessment of mixing efficacy. However, further tests are warranted, particularly using mixtures characterized by significant comminution.

The proposed solution may be used in the homogeneity assessment of multicomponent granular mixtures that are characterized by a significant differentiation. Limitations of this method were not found in relation to the number of mixed components, which is particularly desirable in industrial conditions, e.g. when mixing industrial feeds.

4 Conclusions

- UV-induced fluorescence may be effectively used for the assessment of the content of a key component in a multicomponent granular mixture. The number and variety of ingredients subjected to mixing does not affect the limitations of this method, which is particularly important in industrial conditions.
- No significant differences were observed between the results of the determination of the tracer content using the tested method and the reference method, both for Tinopal and Rhodamine B as the fluorescence-bearing agent.
- Neither of the test substances was shown to be superior to the other in terms of result precision. Thus, both Tinopal and Rhodamine B may be used at specific concentrations (of 0.03% and 0.01%, respectively) to assess the content of a tracer component in multicomponent feed mixtures.
- Larger differences were observed with regard to the homogeneity of mixtures as assessed using both methods. The cut-off threshold of 5% was exceeded for the mixture characterized by the lowest mean grain size. The differences, however, were not statistically significant.
- No impact of the number of mixture components was demonstrated with regard to the study results. On the other hand, further studies are warranted due to the ambiguity of results in relation to the mean size of mixture components.

Conflict of interest: Authors declare no conflict of interest.

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