RESEARCH ARTICLE

Backgrounds of Computer-Aided Assessment of Morphological Structure in Polymer Drugs-Delivery Microcapsules

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Abstract

Microcapsules are widely used as one of the drug delivery means. The paper presents the backgrounds of a method of microcapsules size and shape assessment by computer-aided analysis of microscope images of microcapsules' cross-sections. The method has been elaborated and is practically used to the microcapsules quality control at the Nalecz Institute of Biocybernetics and Biomedical Engineering PAS in Warsaw. Mathematical definitions of parameters describing the size, shape and inner structure of microcapsules are described in the paper. A general scheme of a computer-aided microcapsules quality assessment procedure is presented. Remarks about the limitations of the applicability of the described microcapsules assessment method are also given. On this basis, directions of future works aimed at improvement of the described microcapsules quality assessment method are suggested.

Keywords: drugs delivery, microcapsules, computer- aided image analysis, irregular shape approximation, microcapsules quality assessment

1. Introduction

Among a large variety of drugs delivery methods, microcapsules play a specific role. Having the form of round-shaped vesicles of about $0.005 \div 15$ mm^3 volume, they are suitable for separating some inner from external phases and constitute an environment protecting biologically active materials like: drugs, hormones, enzymes, living cells, microorganisms, etc. to be delivered to a determined locus in a biological tissue or organ. Moreover, microcapsules should play the role of temporal environment for the transferred agents during their performing the planned functions. For this reason, the walls of microcapsules should be permeable to the nutrition on the one, and to the extraction products on the other hand. This means that the walls of microcapsules cannot be made of traditional but rather of specially prepared natural or synthetic, rather porous than cohesive and compact materials. However, the walls of microcapsules should also be mechanically resistant. At last, they should be biologically tolerable for the surrounding tissues and biodegradable after a definite time-interval [1,2,3,4,5,6].

A large group of polymers satisfying the above-mentioned requirements for the microcapsules production can be used [6,7,8,9,10]. Moreover, the production of microcapsules can be based on various technological processes [3,5,11,12]. Keeping the values of geometrical (as well as chemical, biophysical, etc.) parameters of microcapsules within strictly fixed intervals is one of the basic requirements for any technological process. The parameters of interest concern the external and inner size and form of the microcapsules,
as well as the thickness of layers the walls of microcapsules consist of [4,5]. This in turn needs elaboration of adequate and effective methods of measuring the parameters and control of the microcapsules production processes. However, the properties of microcapsules are of random nature which causes a necessity of their description and interpretation in statistical terms [13,14]. Moreover, for technological reasons the information about the size, shape and structure of microcapsules under examination is available from single rather than numerous parallel 2-dimensional (2D) sections. This leads to the necessity of re-interpretation of their 2D microscopic images [13,14,15].
The paper presents some results and experience gained during a multi-year collaboration between the groups of computer and chemistry experts in the Department II of Biomaterials and Biotechnological Systems, c/o the Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences.

The paper is organized as follows: In Sec. 2 the form and inner structure of microcapsules is described. Sec. 3 presents a short review of parameters frequently used for the size and form of microcapsules quantitative assessment.
A scheme of microcapsules quality assessment procedure and remarks on microcapsules' quality assessment accuracy are presented in Sec.4. In Sec.5 concluding remarks and suggestions for future works in computer-aided assessment of microcapsules and control of their production process are given.

2. Shape and structure of microcapsules

Microcapsules have the shapes of 3-dimensional (3D) less or more regular oval objects whose 2D cross-sections in most cases by ellipses (in particular – by circles) can be approximated. The area bounded by the outer and inner contours of a microcapsule belongs to its wall. A typical 2D cross-section of a microcapsule is shown in Fig. 1.

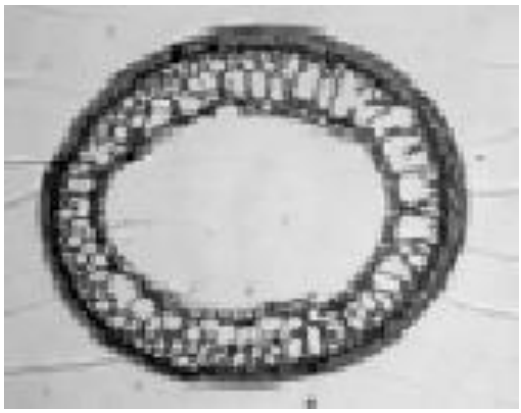

Fig. 1. Typical 2D cross-section of a microcapsule

Both slightly deformed elliptic shapes of the inner and outer contours of the object and inner structure of the wall are remarkable.

The wall consists of compact outer and inner skin layers separated by a porous support layer. The thickness of the layers is not constant around the contours and it depends on the microcapsules production process technology [3]. The process is by its nature stochastic, which means that not only do its individual products (microcapsules) have no ideal shapes and fixed parameters but also their shapes and parameters in large collections are random.

The randomness of size and inner structure of the microcapsules observable on their cross-sections is additionally caused by cross-sections cutting at random angles and levels, as shown in Fig. 2.

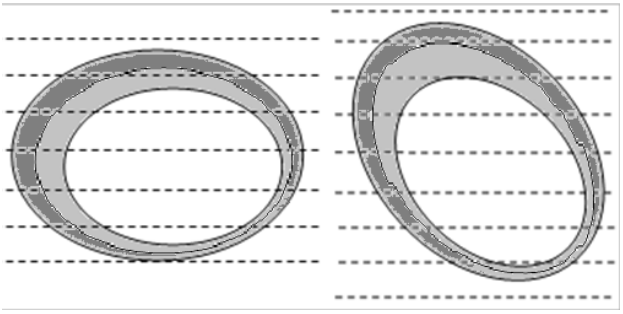

Fig. 2. Size of cross-sections of a microcapsule depending on its position and cut levels

In the case of a cross-section cut close to the top of a microcapsule, the shape of its inner contour may be deformed, as shown in Fig. 3; this makes its approximation by an ellipse incredible or impossible.

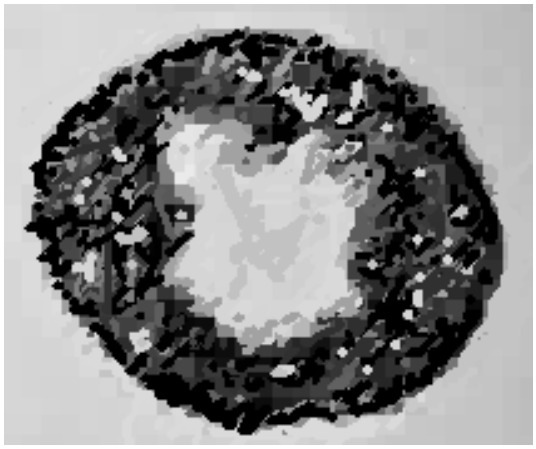

Fig. 3. Example of a defective cross-section of a microcapsule

The defective cross-sections of microcapsules should be automatically detected and removed from the set of observations admitted to further analysis.

Another type of defective microcapsules, caused by technological faults, is shown in Fig. 4. Such microcapsules are of admissible size, defects occurring in their walls and inner area structure.

The disconnection of inner area of a correct-size cross-section can be used as a criterion for selection of this type of defective microcapsules.

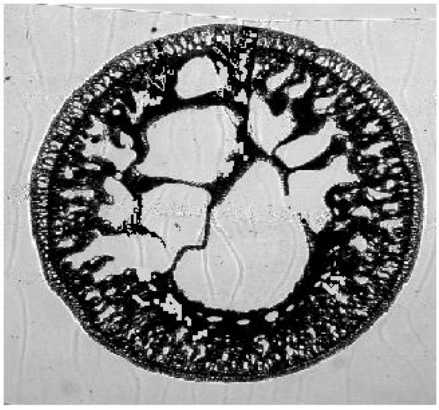

Fig.4. Cross-section of a defective microcapsule caused by technological faults

The images of such defective microcapsules should also be detected, counted and removed from the given set of objects subjected to analysis. Their ratio (percentage) in the set of microcapsules subjected to analysis is an important indicator of the technological process imperfection. The probability distribution parameters describing the microcapsules within a fixed production process should be controlled and kept within admissible limits

Several methods of microcapsules' morphological structure examination can be used, like in particular [13,17]:

- Microcapsule's shape examination under laser beam scattering on the surface;
- Examination of the density distribution of a fluorescent agent penetrating into the microcapsule walls;
- Microcapsule's cross-section analysis under light or electron microscope.

The last method provides the most exact information about the microcapsule's inner morphological structure and its description in strongly defined mathematical terms. However, its main drawback consists in technical difficulty of obtaining several parallel cross- sections of any single examined object. This leads to the necessity of the lacking information indirect reconstruction by adequate mathematical modeling and statistical reasoning.

3. Parameters describing the size and structure of microcapsules

Most parameters describing the size and structure of microcapsules are based on the Euclidean geometry concepts. This concerns both the parameters of the microcapsule as a whole and those describing the structure of its wall. The parameters characterizing the size and general shape of a microcapsule can be divided into three groups:

a) Primary parameters, measurable directly on the cross-sections of a single microcapsule;
b) Secondary parameters, calculated on the basis of the primary parameters;
c) Statistical parameters, based on the primary or secondary parameters in a set of microcapsules taken as samples representing a given technological process.

Assuming that the outer and inner contours visible on a microcapsule's cross-section have the form of mutually separate single closed curves, they can be characterized by the following primary parameters:

L – length of outer contour;
l – length of inner contour;
S – area closed by the outer contour;
s – area closed by the inner contour;
D – diameter of a circle circumscribing the outer contour;
H – maximal span-length;
h – minimal span-length.

The parameters *L, l, S* and *s* in Euclidean geometry need no special comments. The circular diameter *D* roughly describes the size of microcapsule's cross-section, as illustrated by Fig. 5; no information about the shape of the microcapsule under examination is contained in this parameter

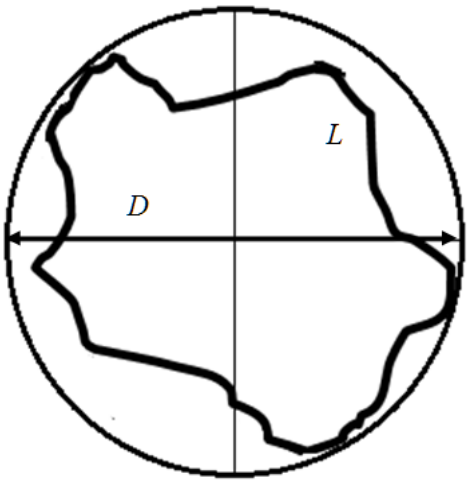

Fig. 5. Circular approximation of a microcapsule's cross-section

Some information about the shape is provided by the span-lengths H and h. The maximal span-length is defined as a maximal distance between two points on the contour, while the minimal span-length means the maximal distance between two opposite points on the contour fixed by a line perpendicular to the maximal-span line. H and h (Feret diameters [18]) denote thus the lengths of sides of a rectangle adjacent outside to the contour and enclosing it as shown in Fig. 6.

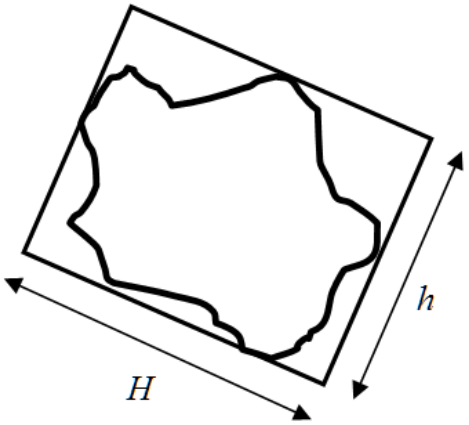

Fig. 6. Contour enclosed by a rectangle

The secondary parameters are used for:

1) Characterization of the shapes of microcapsules;
2) Construction of geometrical models for deeper examination of the microcapsules;
3) Rough indirect evaluation of selected 3-D microcapsule's properties.

The parameters describing the shape of microcapsule or of its interior area are defined as dimensionless ratios, independent of the size of the described object.

Flattening ratio is a basic coefficient characterizing the deviation of outer microcapsule contour from a circular shape:

$$F = \frac{h}{H} \qquad (1)$$

The parameter F takes value 1 if the minimal rectangle enclosing the contour is a square; otherwise it approaches 0 while the contour takes an elongated form. This serves well for a preliminary selection of highly (by flattening or elongation) deformed objects. However, it gives no information about the contour tortuousness connected with microcapsule surface folding. The last property can better be characterized by a relation between the contour length and the area closed by it. The ratio L^2/S in a circle equals $4\pi \approx 12.566$. Therefore, a parameter:

$$k = 12.566 \cdot \frac{S}{L^2} \qquad (2)$$

taking value 1 in circular contours and approaching 0 when the contour becomes more tortuous can be used as an *outer contour circularity coefficient*. A similar *inner contour circularity coefficient* can be defined by replacing the values S and L by s and l, respectively.

A parameter:

$$w = \frac{s}{S} \cdot 100\% \qquad (3)$$

is used as a cross-section *filling coefficient*. In connection with the outer cross-section area S it serves well to discriminate the extreme, close to the tops of microcapsule taken cross-sections.

On similar primary data based parameter

$$\varepsilon = \sqrt{\frac{S-s}{S}} \qquad (4)$$

taking value 1 when $s = 0$ and 0 when $s = S$ describes the *relative thickness of microcapsule walls* with respect to the radius of a circle of area equal S.

The size and inner structure of correct microcapsules can roughly be described by ellipsoidal (3D) model leading to elliptic

(2D) models of the microcapsules' cross-sections.

The size and shape of an ellipse can be described by its major (D) and minor (d) diameters [18]. The ellipse approximate of the outer contour of a microcapsule's cross-section is illustrated by Fig. 7.

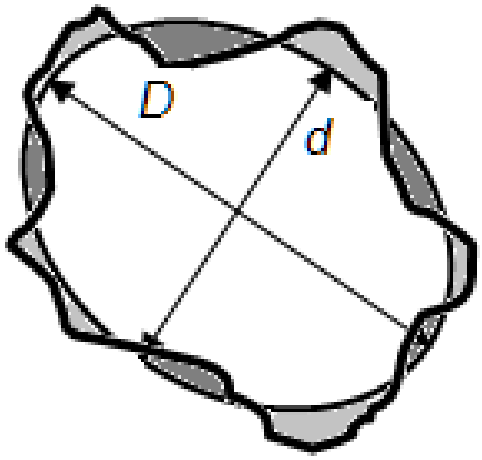

Fig. 7. Microcapsule's contour approximation by an ellipse

The major and minor ellipse diameters have been chosen here so as to make the ellipse area equal to the contour area S:

$$S = \frac{\pi}{4} D \cdot d \ . \qquad (5)$$

On the other hand, D, d and the position of the ellipse imposed on the contour should be chosen so as to minimize a total area of the contour deflections from the ellipse, as shown (in grey) in Fig. 7.

The volume V of an ellipsoid is given by the formula:

$$V = \frac{\pi}{6} D \cdot d \cdot \delta, \qquad (6)$$

δ denoting the length of the third (perpendicular to D and d) diameter of the ellipsoid. Unlike D and d, δ cannot directly be
determined on the basis of 2D cross-section of a microcapsule. However, it is reasonable to admit that a double inequality:

$$d \le \delta \le D \qquad (7)$$

holds in most microcapsules under observation. Establishing δ to be a geometrical mean of D and d, i.e. satisfying the equality:

$$\delta = \sqrt{D \cdot d} \qquad (8)$$

seems to be a reasonable solution of the of 3D information lacking problem. This means that δ should be established as the length of a side of a square whose area is equal to this of a rectangle whose sides are equal D and d.

Under such assumption the formula (6) takes the form:

$$\tilde{V} = \frac{\pi}{6} (D \cdot d)^{3/2} \qquad (9)$$

in which the unknown value δ does not occur explicitly.

Using the ellipsoidal (elliptic) models to evaluate the outer V_{out} and inner V_{inn} volumes of microcapsules makes their classification based on the outer volumes or on the volumetric ratios between the outer to inner volumes possible.

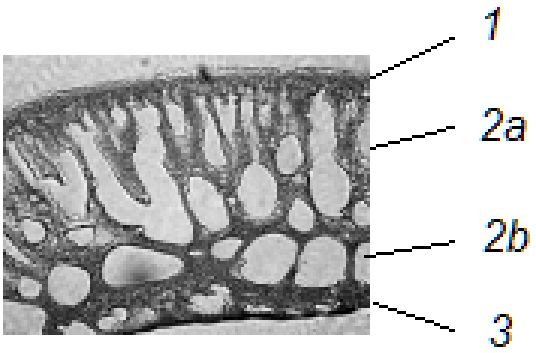

Fig. 8. Inner morphological structure of microcapsule's wall

Another group of parameters to microcapsule's wall morphological structure description is used. A magnified sample of the wall in Fig. 8 is shown. Three main layers can be discriminated there [6]:

1) outer epidermis,

2) intermediate layer,
3) inner skin.

Moreover, the intermediate layer can be divided into two (or more) sub-layers of different porosity characteristics. In practice, the thickness, evenness and porosity of the layers are of interest. However, the borderlines between the layers, and in particular – between the sub-layers (like 2a and 2b in Fig. 8) may not be very clear. For the borderlines plotting various methods can be used. They can mainly be based on detection of the differences of averaged local luminance levels or of selected spectral components intensity [18,19,20].

Regardless of the borderlines plotting method, a problem of microcapsule wall (or layers) thickness profile plotting arises. The thickness profile can be defined as a function $\varepsilon = f(\alpha)$, where α denotes an azimuth of a position vector indicating a current point on the borderline, while ε denotes the wall thickness measured at this point, as shown in Fig. 9.

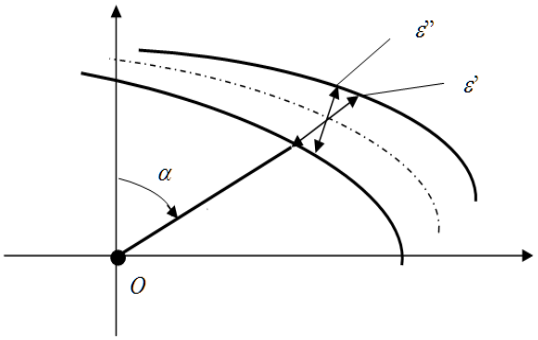

Fig. 9. Scheme of wall thickness measuring

The wall thickness can then be measured along the position vector, as ε' in Fig. 9. However, in this case the result may be overestimated because of the position vector declination from the perpendicular to the borderline. An alternative approach proposed in [6] consists in thickness measuring along a perpendicular to a skeleton line conducted along the layer, as by a dotted line shown in Fig. 9; this thickness has been denoted by ε''. In both the above mentioned cases the microcapsule wall layer can be characterized by four parameters. If $\boldsymbol{e} = [\varepsilon_i]$, $i = 1,2,\ldots,n$, denotes a series of measured thickness values, then the following, based on $\boldsymbol{e}$ parameters can be used to characterize the properties of the layer under examination:

1. the *mean* thickness m_e;
2. the *standard deviation* Δ_e;
3. the *variability* coefficient:

$$\gamma_e = \frac{\Delta_e}{m_e} \qquad (10)$$

4. the *lowest thickness* e_{min}.

The variability coefficient γ_e in a normal microcapsule should be kept below an acceptable, less than 1 level. On the other hand, the lowest thickness should not descend below a fixed threshold level.

Keeping the above-mentioned parameters in proper intervals along full contours of microcapsules' cross-sections may sometimes be too rigorous a requirement. In such case the *evenness* E_e of wall thickness profile can be used [6]. This is defined as the maximal width of a compact angular sector in which the variability coefficient γ_e is kept within the proper interval.

A *general porosity factor* defined as a ratio:

$$G = \frac{S_{por}}{S_{im}} \cdot 100\% \,, \qquad (11)$$

where S_{por} is a total area of pores in a given image area S_{im} , can be used as the main characteristic of inner morphological structure of a microcapsule wall layer [14].

4. Remarks on microcapsules' quality assessment accuracy

High accuracy of the microcapsules characterizing parameters assessment is a necessary condition of sticking to the production process proper standards. Fig. 10

presents a scheme of the microcapsules quality assessment procedure. The errors and/or mistakes made at any stage of this procedure affect the final results of microcapsules' quality assessment.

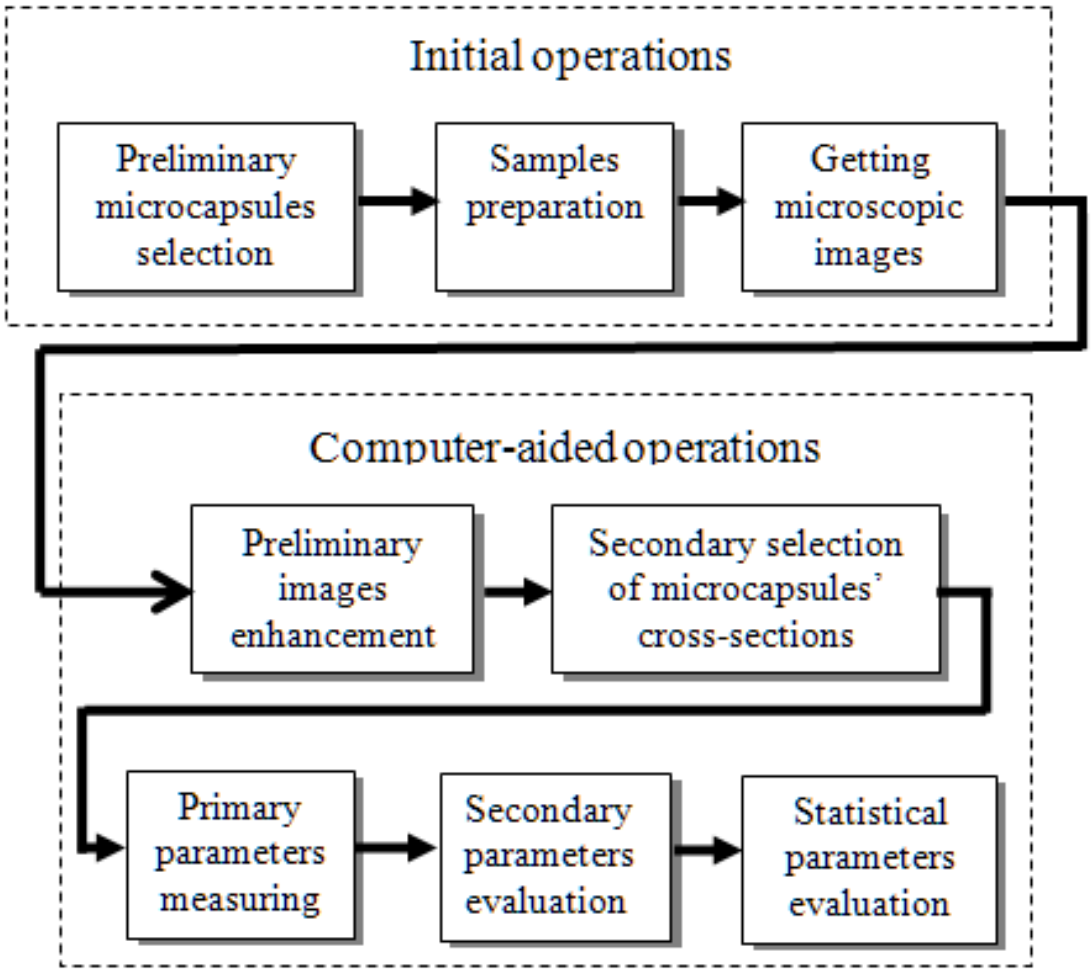

Fig. 10. Scheme of microcapsules' quality assessment procedure

According to this scheme, the following groups of mistakes or errors are possible:

a) At the preliminary microcapsules selection stage, admission of defective objects to further analysis may happen.

b) At the samples preparation stage, the randomness of cutting the microcapsules at different levels and spatial orientations, as shown in Fig. 2, may cause the size of cross-sections not exactly corresponding to the real size of the objects under examination. This problem was more deeply analyzed in [15].

c) The cross-sections of microcapsules selected from the objects obtained in given production processes should be collected in series corresponding to given technological processes. The sets of microscope preparations destined for computer analysis should thus contain the objects obtained in a strongly defined technological regime of microcapsules production. Neglecting this requirement can make the production process

optimization based on the microcapsules' quality assessment impossible.

d) The series of microscope images should be subjected to a preliminary computer-aided image enhancement process consisting in their denoising, contrast improving and highlighting the morphological details [18,19]. However, such operations usually lightly change the size and shape of blurred visual objects. Consequently, the evaluated size and shape after such operations may not quite exactly reflect those of the real objects under examination. It is thus desired that the image enhancement procedures are fixed at least within a given series of the images under examination.

e) The secondary selection is aimed at rejecting the images of cross-sections that after the former operations are qualified as still representing the defective microcapsules. Incorrect decisions made at this stage directly influence the statistics describing the quality of microcapsules production process.

f) The continuous lines (L, l etc.) are approximated in a computer by sequences of pixels. Consequently, their lengths are given by sums of lengths of shorter discrete segments. However, those segments can be defined and calculated in several ways according to the type of geometry used: 1) discrete 4-connective, 2) discrete 8-connective, 3) continuous (Euclidean). The difference between them in Fig.11 is illustrated as distances between a pair of black-marked pixels.

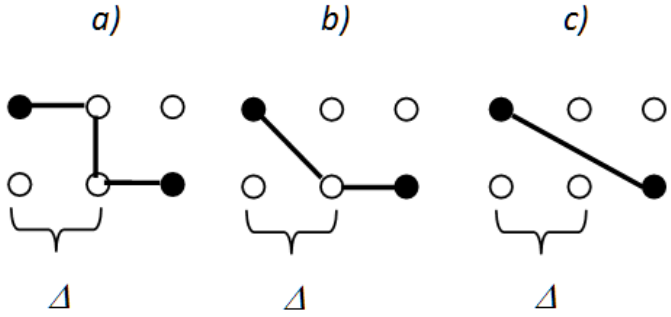

Fig. 11. Various distance measures in: *a)* discrete 4-connective; *b)* discrete 8-connective; *c)* Euclidean (continuous) geometry.

The calculated values in this case are equal: *a*) 3Δ in discrete 4-connective, *b*) 2.414Δ in discrete 8-connective, *c*) 2.236Δ in continuous (Euclidean) geometry, Δ denoting a distance between adjacent pixels. Some other effects of discrete geometry influence on the form of small geometrical objects have been described in [21].

Consequently, the method of computer-aided distance measuring in all measurements within a given series of images should be unchanged, otherwise the results may be incomparable.

a) The given by the formulae (2) or (5) secondary parameters characterizing the shape of microcapsule cross-section hold only if the lengths in the sense of Euclidean geometry have been expressed.

The borderline between the microcapsule wall's sub-layers can be demarcated with the lower accuracy the lower is the difference between the porosity levels or the morphological spectra of the sub-layers.

The inaccuracy concerning the parameters of the 2D cross-sections' extrapolated on the corresponding 3D default objects can't be completely reduced, the extrapolation being based not on additional information but on arbitrary assumptions.

b) Microcapsules quality parameters corresponding to the time-ordered series of samples taken within a given production process provide information about the technological regime stability. The time-variations of the series of parameters are then the more easily detected the lower is the statistical error of the parameters evaluation.

5. Conclusions

A combination of microscope- and computer-aided image processing and analysis technologies is a powerful tool of microcapsules' quality assessment. It provides a possibility of microcapsules inspection for keeping their size and morphological structure within required limits. Moreover, the approach being based on mathematical concepts and models of parameters describing the form of microcapsules can be effectively automated. Most of the above-mentioned computer-aided image enhancement and parameters calculation procedures in principle can be performed by using a standard mathematical software. However, more effective results can be reached by using specialized software (like the MeMoExplorer system described in [15]).

Effective automation of the microcapsules quality assessment needs also a careful preparation of the microcapsules cross-sections for examination. A knowledge of the inherent limitations of the computer-aided image processing methods is also necessary for correct interpretation of the results of microcapsules' quality assessment. For making the assessment methods more effective elaboration of more perfect mathematical models describing the shape and inner morphological structure of microcapsules is desired. Aspiring to accelerate the microcapsules assessment procedure up to making it able to be used to real-time control of the production process seems also to be desirable.

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