Scanning electron microscopy and X-ray energy dispersive spectroscopy – useful tools in the analysis of pharmaceutical products

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Abstract. The quality of the drug, its purity and identification of degradation products provide the highest quality of pharmaceutical products. The energy dispersive spectroscopy (EDS) method analyses the percentage of each element form as well as their distribution, and morphological characteristics of the drug form. We analysed the usefulness of EDS method in testing orally disintegrating tablets (ODT) with trimetazidine hydrochloride with high resolution scanning electron microscopy (SEM, SUPRA25 Carl Zeiss company) with spectrophotometer equipped with an X-ray energy dispersion (EDAX Company). The samples of the analysed tablets were imaged after applying conductive layers of gold on their surface. In the EDS analysis the compositions of each sample of the obtained tablets were observed to be virtually identical. The differences in the content of carbon and oxygen came from differences in the composition of particular tablets. The presence of gold in the composition resulted from the sputtering the surface of tablets with gold during the analysis. Knowing the composition of the tablet, SEM-EDS method helps to locate and identify the impurities and degradation products of the compounds, leading to a better understanding of the mechanisms of their formation.

Key words: scanning electron microscopy, energy dispersive spectroscopy, SEM-EDS analysis, pharmaceutical systems

1. Introduction

The quality of the drug, its purity and identification of degradation products provide the highest quality during preparation of pharmaceutical products. Number of novel techniques, i.e. Raman spectroscopy, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) or microtomography are increasingly used in the analysis of finished pharmaceutical products [1, 2]. However, these techniques are still not a standard procedures in the pharmacy research.

The EDS detectors are components of scanning as well as of transmission microscopes. They enable qualitative and semi-quantitative identification of chemical elements in a variety types of material due to the X-rays detection of elemental composition of the tested sample. During the analysis two types of the radiations are examined: continuous radiation which forms the background of the measurement
and the characteristic radiation of a specific wavelength and energy amount. SEM-EDS method allow to know the composition of elements as well as their percentage in the analyzed pharmaceutical form. SEM-EDS can also locate and identify the impurities and degradation products of the compounds in the drug form, leading to a better understanding of the mechanisms of their formation [3, 4].

The aim of the present study was testing ODT tablets containing trimetazidine hydrochloride as a model active substance using EDS method. Trimetazidine is an anti-ischemic drug with short half-life (6 ± 1.4 h). The orally disintegrating tablets, after placing them on the tongue, should disintegrate to granules or powder, which are then easy to swallow during three minutes according to Pharmacopoeia Europaea [5] or up to 30 seconds according to Food and Drug Administration [6]. The ODTs are clinically attractive due to the possibility of achieving the expected therapeutic effect [7].

2. Material and methods

2.1. Preparation of the tablets

During the preformulation phase of the study we prepared placebo tablets (without trimetazidine) and tablets with 20 mg trimetazidine hydrochloride (formulations ODT 1, ODT2; Table 1).

Table 1. Compounds of the ODT preformulations to select the manufacturing method

| COMPOUNDS                        | FORMULATIONS                                      | ODT 1A (direct compression, starch) | ODT 2A (direct compression, Avicel) | ODT 1B (wet granulation, starch) | ODT 2B (wet granulation, Avicel) |
|----------------------------------|---------------------------------------------------|-------------------------------------|-------------------------------------|----------------------------------|----------------------------------|
| Trimetazidine hydrochloride      | P [mg] T [mg]                                     | P [mg] T [mg]                       | P [mg] T [mg]                       | P [mg] T [mg]                    | P [mg] T [mg]                    |
| Lactose monohydrate              | 48 28                                            | 48 28                               | 48 28                               | 48 28                            | 48 28                            |
| Sorbitol                         | 25 25                                            | 25 25                               | 25 25                               | 25 25                            | 25 25                            |
| Starch                           | 25 25                                            | - 25                                | - 25                                | - 25                             | 25 25                            |
| Microcrystalline cellulose       | - 25                                             | - 25                                | - 25                                | - 25                             | - 25                             |
| Talcum                           | 2 2                                              | 2 2                                 | 2 2                                 | 2 2                              | 2 2                              |
| Starch mucilage (binder)         | - 25                                             | - 25                                | - 25                                | - 25                             | - 25                             |
| Total weight                     | 100mg 100mg                                      | 100mg 100mg                         | 100mg 100mg                         | 100mg 100mg                      | 100mg 100mg                      |

P - placebo tablets; T - tablets with active substance

After selection of the preparation method of ODT tablets, four formulations were prepared. Each formulation contains 20 mg of trimetazidine hydrochloride and 25 mg of sorbitol. In turn, the following compounds differed between the formulations:

- **ODT 1** – 28 mg of lactose, 25 mg of starch and 2mg of talcum
- **ODT 2** – 28 mg of lactose, 25 mg of microcrystalline cellulose and 2mg of talcum
- **ODT 3** – 38 mg of lactose, 15 mg of crospovidone and 2mg of magnesium stearate
- **ODT 4** – 43 mg of lactose, 10 mg of croscarmellose sodium and 2mg of magnesium stearate.

The total weight of ODT tablets was 100 mg. All tablets were pressed using the force of 8kN with Erweka AR 400 tablet press machine.

2.2. Scanning electron microscopy with X-ray energy dispersion

The high resolution scanning electron microscopy (SEM, SUPRA25 Carl Zeiss company, Germany) with spectrophotometer equipped with an X-ray energy dispersion (EDAX Company) was used. Each sample was covered with a gold layer using Sputter Coater 108auto (Cressington) [8]. SEM images were obtained at an acceleration voltage from 5 to 10 kV. EDS analysis as an extension of SEM was used in the mode of semi-quantitative detection to detect the element composition of the prepared tablets and the acceleration voltage used was 10 kV.
3. Results

3.1. Selection of the preparation method of the tablets

In the preformulation processes the disintegration time measured in artificial saliva (pH 5.8) was shorter for formulations containing Avicel prepared with direct compression than for tablets with wet granulation step. Tablets with Avicel made with the use of direct compression disintegrated faster than tablets with starch. Surprisingly, the tablets with Avicel compressed after wet granulation disintegrated slower than those with starch made with wet granulation step. It was observed that tablets made of formulation ODT 2B (Avicel) disintegrated into the granules first. In turn, tablets from formulation ODT 1B (starch) showed more homogenic form after disintegration. It was also demonstrated that addition of active substance resulted in longer disintegration and wetting times compared to placebo tablets in case of ODT 1A, ODT 2A and ODT 1B formulations. In case of friability, all series of tablets met the requirements of the FP VII - none of the analyzed tablets showed loss of weight more than 1%.

Considering the results of preformulation analysis, direct compression as a method of receiving ODT tablets was then performed and the produced tablets analyzed to examine their disintegration time. It was observed that the shortest disintegration time was observed for ODT tablets with crospovidone while the longest one – for ODT with starch.

3.1.1. SEM and EDS analysis results

Figure 1 shows SEM analysis of the samples of the ODT tablets. Figure 1a presents the surface of the tablet with starch made with wet granulation process – visible granules as well as some amount of powders are shown, which may indicate partial destruction of the granules during the compression. Figures 1b and 1c show the porous surface of the ODT tablets containing Avicel and crospovidone, which is an important parameter for ODT tablets since it influence the tablet disintegration time.

![SEM images of ODT tablets](image)

**Figure 1.** The example images of the surface of ODT tablets (a-ODT with starch made with wet granulation; b-ODT with Avicel and direct compression; c-ODT with crospovidone and direct compression). In all cases the magnification was 1.00 KX

In the EDS technique the composition of two samples of each tablets was analysed. Figure 2 shows the imaging results of EDS analysis. In all analyses some amount of gold was found in the samples which is the effect of the sputtering with gold on the surface of the samples.
Figure 2. The results of EDS analysis (a-ODT with starch made with wet granulation; b-ODT with Avicel; c-ODT with crospovidone; d - ODT with croscarmellose sodium)

Table 2 presents the amounts of the elements. Noticeable, but insignificant differences in the content of carbon and oxygen arise from different quantity of excipients used for preparation of the particular type of ODT tablet. The presence of the silicon and magnesium results from talcum, which was used as an anti-adhesive ingredient in the tablets with starch and Avicel. In turn, the chlorine is from trimetazidine hydrochloride.

Table 2. The comparison of the percentages of basic elements between all analysed formulations

| Element | ODT 1 (Starch) [%] | ODT 2 (Avicel) [%] | ODT 3 (Crospovidone) [%] | ODT 4 (Croscarmellose sodium) [%] |
|---------|--------------------|--------------------|--------------------------|----------------------------------|
| C       | 58.28              | 60.08              | 62.02                    | 62.40                            |
| O       | 34.24              | 31.72              | 29.69                    | 30.38                            |
| Cl      | 03.65              | 03.83              | 04.47                    | 04.63                            |

4. Discussion

The surface of particles, tablets or capsules can be analyzed with EDS, however there is little data available so far in the topic [9, 10]. The EDS technique also helps in analysis of contamination due to the presence of particulate matter in pharmaceutical products. Such impurity may lead to quality and safety problems
or even to suspension of the production [11]. The elements with low atomic number have a lower X-ray yield than the elements with high atomic number hence, there may be problems with their detection. The EDS detector is not very sensitive to C, H, O, and N, which are major elements of active substances. In turn, the excipients used during the preparation of a drug form frequently contain Ca, P, Ti, Mg or Si [12]. In the present study we found that chemical composition of scanned sample of the tablet surface did not deviate from the content of powders taking for the obtaining of the tablets. We have not observed any additional elements, which may indicate some impurity of the product. The area of each chemical compound is scanned with the EDS directly thus it is observed that measurement with EDS is much faster and more accurate than confocal Raman [9]. On the other hand, this area is rather small, up to 100 µm² vs. several mm² in Raman scanning. Therefore, it appears that a large number of EDS scans at different locations on the surface of drug form, e.g. tablets may give more accurate surface imaging [9].

Previously, the study of drug distribution with EDS analysis in tablets containing paracetamol was performed [9]. The authors observed that tablets made from the pre-mixed extruded formulations had better uniformity and distribution of API than tablets from hot-melt extruded formulations or tablets made with direct compression. Additionally, the amount of each element was in agreement with the expected values. Seitavuopio et al. [13] observed changes on the tablets surface roughness during the coating as well as the presence of magnesium stearate and titanium dioxide with SEM-EDS. It was demonstrated that the magnesium signal disappeared within 15-30 minutes, which meant that the tablet was homogeneously covered during the coating [13]. In another study, the migration of the water soluble components between the coatings was observed with EDS method and sodium as representative ion in prednisolone pellets coated with Eudragit L30 D-55. The authors demonstrated that sodium was first concentrated in the inner coat and during the dissolution it migrated into the outer coat [10].

5. Conclusions

The present study shows that EDS analysis may give useful information in pharmaceutical studies by verifying the presence or lack of impurities in the examined solid oral dosage form as well as the manufacturing process.

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