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Evaluation of usefulness of CA and PCA for identification of incompatibilities in the pharmaceutical phase

Ocena przydatności CA i PCA w identyfikacji niezgodności w fazie farmaceutycznej

Thermal analysis is used in the pharmaceutical industry as a reliable technique for quality control and for the development of new pharmaceutical formulations [2, 5, 9, 15, 16]. During the manufacture of a commercial drug, active components are mixed with excipients [3, 4, 11, 12]. The latter sometimes cause variation in the physicochemical properties of the active component. Chemical interaction can lead to degradation of the active ingredient, thereby reducing the amount available for therapeutic effect. Moreover, the reaction products may diminish safety of the therapy. On the other hand, physical interactions can affect the rate of dissolution, uniformity of the dose or ease of administration.

Instrumental analytical techniques frequently used in a study of the solid drugs, e.g. differential scanning calorimetry (DSC) and thermogravimetry (TG) generate in a relatively short time large sets of measurement data which describe not only physicochemical properties of the samples under study, but also provide information about their chemical composition [19, 20]. The proper interpretation of multidimensional sets of data appeared to be difficult in realization, mainly because of complexity of processes taking part in the systems with the participation of drugs. Due to this reason, in the last decade the interest of pharmaceutical sciences in the use of statistical multivariate techniques for studying of huge databases obtained after analysis of drugs by instrumental techniques significantly increased [18].

Regarding the above, the objective of the study was to examine if two of the most frequently used multivariate statistical techniques such as cluster analysis (CA) and principal component analysis (PCA) can be used as the supporting techniques for identification of the incompatibility occurring in the binary mixtures of acetazolamide, atenolol, baclofen, hydrocortisone and piroxicam with the excipients such as lactose, mannitol, methylcellulose, starch, magnesium stearate and talc. For the analysis purpose, the binary physical mixtures of drug to excipent in the molar or mass ratios of 9:1, 7:3, 1:1, 3:7 and 1:9 were prepared. Each drug, excipient and physical mixture were studied with the use of the DSC, DTA, TG and DTG techniques as well as hot-stage microscopy (HSM) and infrared spectroscopy (IR), making the instrumental data set for multivariate calculations.

MATERIAL AND METHODS

The following drugs and excipients were used in the studies (manufacturers are given in the parentheses): acetazolamide (Polfa, Warsaw), atenolol (Polpharma, Starogard Gd.), baclofen (Polpharma, Starogard Gd.), hydrocortisone (Pharma Cosmetic, Krakow), lactose (BUFA B.V.,

Holland), magnesium stearate (Faci Carsco, Italy), mannitol (POCh, Gliwice), methylcellulose (Shin-Etsu Chemical Co., Japan), piroxicam (GlaxoSmithKline Pharmaceuticals, England), starch (POCh, Gliwice), talc (Koeln, Italy). All compounds were analyzed without further purification.

The binary physical mixtures of drugs and excipients (at the molar or mass ratios of 9:1, 7:3, 1:1, 3:7, 1:9) were prepared by mixing adequate amounts of both substances in a porcelain mortar. Compounds with similar molar masses were mixed at the molar ratios, whereas these differing significantly in molar masses were mixed at mass ratios.

DTA, TG and DTG curves of thermal decomposition of the samples under study were performed using a derivatograph (MOM, model OD-103, Hungary). 100–200 mg samples placed in four flat-bottomed platinum pans set were heated in an air at a heating rate of 5ºC·min^{-1} up to the final temperature of 700ºC. As the reference material α-Al_2O_3 was employed.

DSC was performed for drugs and excipents alone and for physical mixtures of these components. Samples under study of approximately 4–5 mg were accurately weighed (±0.01 mg) and encapsulated in 40 μl flat-bottomed aluminium pans with crimped-on lids. DSC analyses were carried out with a heat-flux Mettler Toledo instrument (model DSC 822^e, Switzerland) with a liquid nitrogen cooling system (Dewar vessel). Measurements in the temperature range from 20 to 300ºC were obtained at a scanning speed of 10ºC·min^{-1} under dynamic purging nitrogen at the flow rate of 70 mL·min^{-1}. Indium and zinc standards were used to calibrate the DSC cell. For analysis of the DSC scans the STAR^e software were used.

The melting points were obtained using Boëtius hot-stage microscopy (Carl Zeiss Jena, Germany) at the heating rate of 4ºC·min^{-1} from room temperature up to 350ºC.

Samples for recording the IR spectra of the tested substances and their physical mixtures were prepared as KBr pellets. Each pellet was prepared from 1 mg homogenized substance and 100 mg spectroscopy-grade KBr (Merck, Germany). The infrared spectra were recorded at room temperature in the 4000–200 cm^{-1} region on a Carl Zeiss Jena instrument (Specord, model M-80, Germany). Atmosphere was employed as the background. The analysis of IR spectra was made by using the Spectra Manager software.

Calculations. Two multivariate statistical techniques, cluster analysis (CA) and principal component analysis (PCA), were applied for interpretation of the results. Matrix of the data X with dimensions n · p, where n is a number of objects (rows) while p is a number of variables (columns), was the starting point for calculations. In the matrix drug and excipient alone and the physical mixtures of both components were used as the rows. Columns were the thermal parameters obtained from the TG and DTG curves for the analyzed samples. Statsoft Statistica release 7.1 (StatSoft Inc., USA) was used to perform cluster and principal component analyses.

RESULTS AND DISCUSSION

Structural formulas of the drugs are given in Fig. 1, whereas results of the thermal decomposition of drugs and excipients are compiled in Table 1. Interpretation of these data allowed to find out that the thermal decomposition of all of the compounds under study proceeds in three stages. The first stage comprises the range of temperatures, in which loss of mass on the TG and DTG curves was not registered. The first stage is present only in the case of compounds, in which phase transition of the first degree was observed, e.g. melting or polymorphism. These processes are confirmed by narrow and sharp ended endothermic peaks on the DTA curves.

Table 1. Phase transformations and thermal processes occurring in drugs and excipients during heating

Drugs and excipients	Literature data ºC	Experimental data ºC
Acetazolamide	260 melting [10]	250 melting with evaporation and decomposition
Atenolol	146–148, 150–155 melting [10] 152–155 melting [1] 152–156.5 melting [17]	153 melting
Baclofen	195–200 melting [14]	198–205 melting
Hydrocortisone	216 melting with decomposition [14] 214 melting [10]	195–215 melting with decomposition
Piroxicam	polymorphic transitions [10] 196–198 needlic structures 199–201 cubic structures	200–207 polymorphic transitions
Lactose	103–120 water evaporation 201–202 monohydrate α structure 223 anhydrous α structure 252.2 anhydrous β structure [8]	137 formation of water 157 partial carmelization 217–235 melting
Mannitol	166–168 melting [8] 165–170 melting [1] 164–169 melting [17]	165–170 melting
Methylcellulose	190–200 melting 225–230 combustion [8]	287–306 decomposition 310–320 combustion
Starch	no data	63 swelling point 72 gelating point 236–237 melting with decomposition
Magnesium stearate	117–150 melting [8]	160–170 melting
Talc	850 – ignition point [1] > 800 difficult melt [7]	25–350 no changes

In the second stage of decomposition, several dozen percent mass loss was observed. At this stage, intermediate products of decomposition, are formed. The chemical constitution and structure of these products could not be determined due to the multidirectional course of thermal destruction of organic matter. The second stage was divided into several substages. This is caused by the parallel and consecutive reactions of the formation of intermediate products and by overlapping of their thermal effects.

The third stage includes the final compound decomposition which is based on combustion of high temperature carbonization residues. At this stage for each compound a strong exothermic effect on the DTA curve and a small peak on the DTG curve reflecting the mass loss on the TG curve can be observed.

Interactions between the studied components in the binary mixtures were detected by comparing thermograms and IR spectra of pure drugs and excipients with thermograms and IR spectra of their physical mixtures in different ratios. The thermal analysis is completed with hot-stage microscopy studies.

DSC and DTA allowed evaluation of incompatibilities according to appearance, shift or disappearance of peaks and/or variations in the corresponding enthalpies. Variations in the peak shape, onset and peak temperatures as well as in the peak width and height also provided valuable data. They can reveal the formation of new bonds. Shift onset of decomposition of drugs to lower temperatures reflects the influence of one component on the stability of another. Taking all of the above into consideration, based on the analysis of DTA, TG and DTG curves showed in Fig. 2, it seems very probable that incompatibility in the mixtures of acetazolamide with methylcellulose is present.

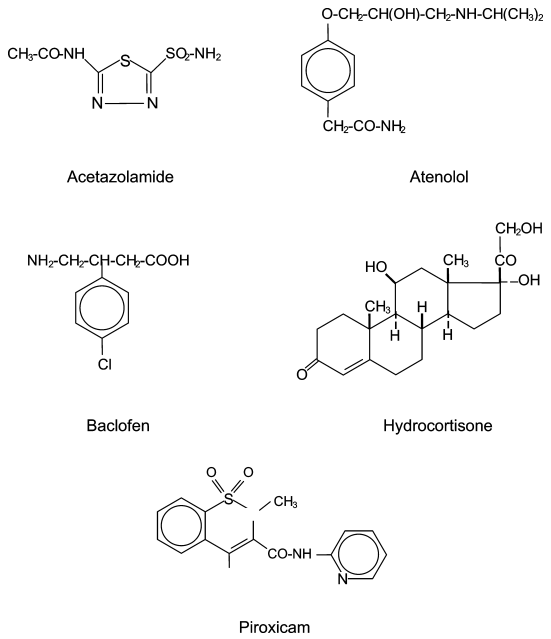

Fig. 1. Chemical formulas of the drugs under study: acetazolamide, atenolol, baclofen, hydrocortisone and piroxicam

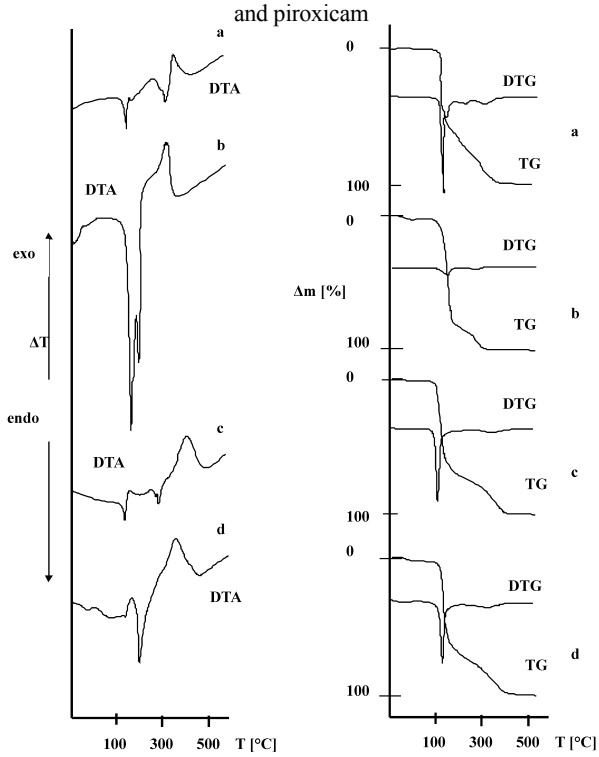

Fig. 2. DTA, TG and DTG curves of: (a) acetazolamide, (b) methylcellulose, and the physical mixtures of acetazolamide with methylcellulose in the mass ratios of: (c) 1:1 and (d) 1:9

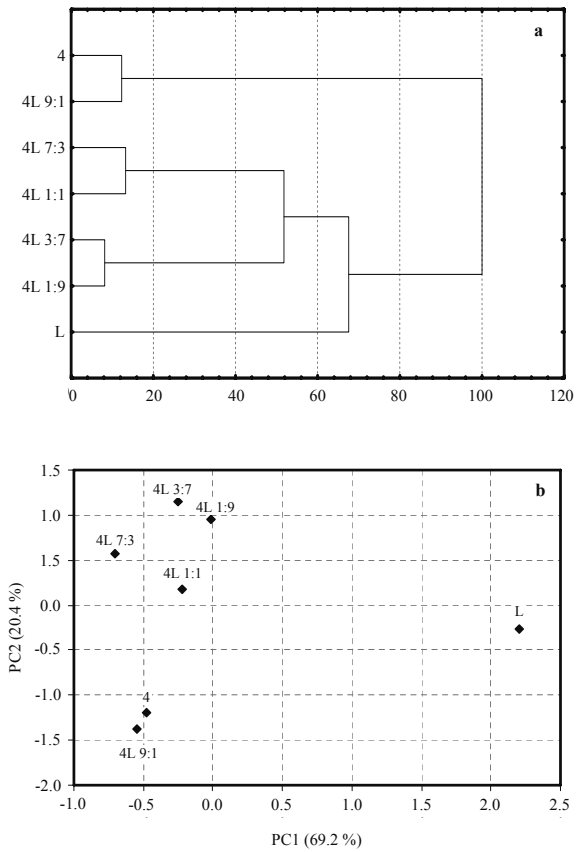

Fig. 3. CA dendrogram (a) and PCA score plot for the first two components (b) for piroxicam denoted as number 8, starch denoted as letter M, and the physical mixtures of both components mixed in the mass ratios of: 9:1, 7:3, 1:1, 3:7 and 1:9

IR was also used as an assisting technique to thermal methods. The chemical interactions between drugs and excipients are proved with the help of IR spectroscopy by the following important characteristics: appearance of new IR absorption bands, broadening of bands and alteration in intensity.

For chemometric evaluation of the thermoanalytical results, two multivariate methods (pattern recognition methods): cluster (CA) and principal component (PCA) analyses were used [6, 13]. Their valuable feature is that they are capable of doing the correct interpretation of the measurement data and to obtain the maximally useful information from them.

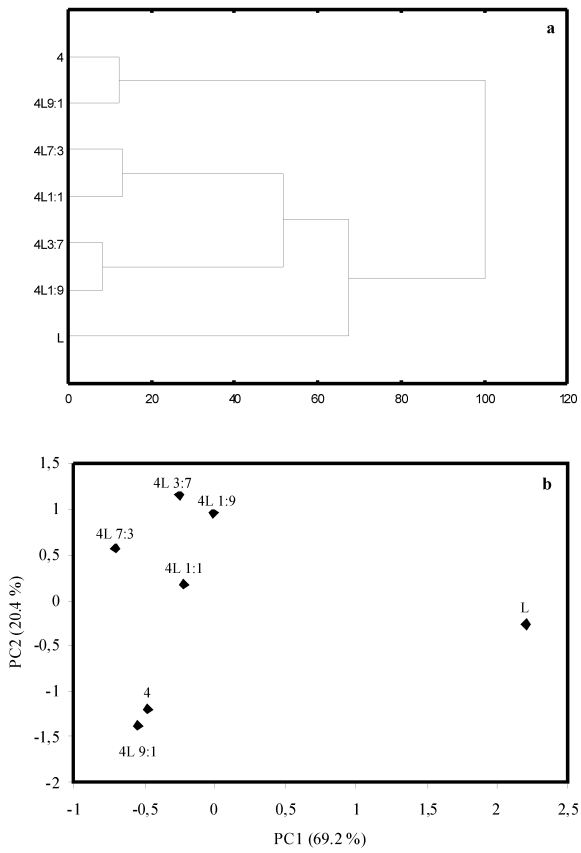

Fig. 4. CA dendrogram (a) and PCA score plot for the first two components (b) for acetazolamide denoted as number 4, methylcellulose denoted as letter L, and the physical mixtures of both components mixed in the mass ratios of: 9:1, 7:3, 1:1, 3:7 and 1:9

The purpose of CA is to arrange objects into groups of similar characteristics. The calculations were based on the measurements of similarity between objects and clusters of objects. Similarity was defined as Euclidean distance in property space in which the objects were represented by points and clusters by groups of points. From the multitude of algorithmic approaches to clustering, one of the most popular hierarchical agglomerative type algorithm, the Ward's method, was employed. The agglomerative approach begins with a structure of n clusters, one per object, which grows a sequence of clusters until all n patterns are in a single cluster.

Contrary to CA, PCA can be used to reduce dimensionality of the complex multivariate data by deriving a new set of variables, noncorrelated with each other and representing a certain quantity of features of the data set. All of them have the variance of one and they contain a certain part of the total variance of data set expressed by their eigenvalues. New variables labeled as principal components (PC) were calculated as columns in two new matrices P and W. New matrix P reflects the main relations among objects and makes classification of the samples under study possible according to their physicochemical properties, whereas matrix W illustrates the main relations among variables and enables selection of the key thermal and spectral parameters, which makes the best classification of the studied samples.

Results of the CA and PCA calculations are graphically presented in Figs. 3 and 4. Analyzing the similarity between drugs, excipients and physical mixtures of both components (Fig. 3A and Fig. 4A), as well as of distribution of these samples in two-dimensional space (Fig. 3B and Fig. 4B), it was found that localization of most mixtures in one cluster distinctly separated from drug and excipient proved that incompatibility in these mixtures takes place. In the case of absence of interactions, the whole dataset could be modeled with one principal component, explaining almost whole variance. The mixtures would then be placed along the straight line in PC1 vs. PC2 plot. In our case, only about 70% of variance is modeled by PC1, whereas most of remaining variance is modeled by PC2. Additionally, the samples do not form a straight line, but visible two-dimensional trend. This proves the interactions and suggests that at least 20% of whole information comes from them.

CONCLUSIONS

DSC method combined with simultaneous DTA and TG, infrared spectroscopy and hot-stage microscopy analyses allows to evaluate the incompatibility or compatibility between both components of the binary mixtures. Based on these results, it can be shown that the physicochemical incompatibilities in the binary mixtures of acetazolamide with lactose, mannitol, methylcellulose, starch and baclofen with lactose, magnesium stearate are probable.

Results of the statistical analysis showed that CA and PCA are very helpful in the interpretation of the thermoanalytical data, besides in all doubtful cases these methods gave, proper interpretation of the results.

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SUMMARY

The objective of the study was an attempt to examine the usefulness of the most frequently used multivariate techniques in the pharmaceutical analysis such as cluster analysis (CA) and principal component analysis (PCA), as the supporting methods for identification of the physicochemical incompatibility occurring between drugs and excipients. The binary physical mixtures of acetazolamide, atenolol, baclofen, hydrocortisone and piroxicam with the excipients such as lactose, mannitol, methylcellulose, starch, magnesium stearate and talc, mixed in the molar or mass ratios of 9:1, 7:3, 1:1, 3:7 and 1:9, were studied. The possibility of incompatibilities between both components of the mixtures under study was investigated based on the results obtained with the help of differential scanning calorimetry (DSC), differential thermal analysis (DTA), thermogravimetry (TG) as well as infrared spectroscopy (IR) and hot-stage microscopy (HSM). The obtained results were interpreted with the help of chemometric techniques. Based on these results it can be shown that the physicochemical incompatibilities in the binary physical mixtures of acetazolamide with lactose, mannitol, methylcellulose, starch and baclofen with lactose, magnesium stearate are probable. It was confirmed by the results of the statistical analysis which showed that CA and PCA techniques are very helpful in the interpretation of thermoanalytical data.

STRESZCZENIE

Celem pracy była próba oceny użyteczności najczęściej stosowanych w farmacji technik analizy wielowymiarowej, takich jak analiza skupień (CA) i analiza głównych składowych (PCA), jako metod wspomagających identyfikację niezgodności fizykochemicznych między substancjami leczniczymi i pomocniczymi. Analizie poddano dwuskładnikowe mieszaniny fizyczne substancji leczniczych: acetazolamidu, atenololu, baklofenu, hydrokortyzonu i piroksykamu z wybranymi substancjami pomocniczymi, takimi jak laktoza, mannitol, metyloceluloza, skrobia, stearynian magnezu i talk, zmieszanych w stosunkach molowych lub masowych: 9:1, 7:3, 1:1, 3:7 i 1:9. Możliwość wystąpienia

niezgodności fizykochemicznych między oboma składnikami badanych mieszanin oceniano na podstawie wyników analiz technikami różnicowej kalorymetrii skaningowej (DSC), różnicowej analizy termicznej (DTA), termograwimetrii (TG) oraz spektroskopii w poczerwieni (IR) i termomikroskopii (HSM), a następnie uzyskane wyniki poddano interpretacji za pomocą technik chemometrycznych. Na podstawie uzyskanych wyników można stwierdzić, iż w dwuskładnikowych mieszaninach fizycznych acetazolamidu z laktozą, mannitolem, metylocelulozą i skrobią oraz baklofenu z laktozą i stearynianem magnezu najprawdopodobniej występują niezgodności fizykochemiczne. Zostało to potwierdzone za pomocą wyników analizy statystycznej, która wykazała, że techniki CA i PCA są bardzo pomocne w interpretacji danych termoanalitycznych.