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Research paper

Interactions between bendroflumethiazide and water soluble polymers. II. Factors promoting drug-polymer association

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Abstract

In stressed solid dispersions of bendroflumethiazide in polyethylene glycol 6000, in polyvinylpyrrolidone K 25, and in Poloxamer 188, associates are postulated to be formed. The effects of the factors, concentration, process of manufacture, pH, relative humidity, and temperature, were investigated by factorial design studies. After about 25 days, a steady state in associate concentration was observed. The formation of these associates is promoted mainly by low percentiles of the guest, high temperature close to the melting point of the solid dispersions, and relative humidity. © 1997 Elsevier Science B.V.

Keywords: Solid dispersion; Associate; Factorial design; Polyethylene glycol; Polyvinylpyrrolidone; Poloxamer; Bendroflumethiazide

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1. Introduction

In Part I of this work [1], associates of bendroflumethiazide (BFMT) and water soluble polymers have been postulated to be formed in solid dispersions during storage under climatic stress. These associates were quantified with an acceptable experimental error by HPLC as the difference between the molar sum of the detected compounds and the initial amount of BFMT.

The aim of this study was to quantify the effects of important factors by factorial-design analysis and by kinetic studies. The systems BFMT/polyvinylpyrrolidone K 25 (PVP K 25), BFMT/polyethylene glycol 6000 (PEG 6000) and BFMT/poloxamer 188 (Poloxamer 188) were investigated. Solid dispersions in PVP K 25 of the main degradation product of BFMT, 5-trifluromethyl-2,4-disulfoamoylaniline (TFSA), were employed in kinetic studies, since TFSA forms similar associates but does not further degrade.

2. Materials and methods

BFMT, PVP K 25, PEG 6000 and Poloxamer 188 were supplied and used as described in Part I [1]. TFSA was obtained by hydrolysis of BFMT in 20% NaOH and further purification. The content of the purified substance was determined by HPLC using a BP-CRS as standard and was found to be 99.6 ± 0.5 ($n = 6$). All other reagents were of analytical or HPLC grade.

Solid dispersions of BFMT were prepared according to the solution method in Part I [1]. All solid dispersions were amorphous by X-ray diffraction.

To obtain a defined pH in the solid dispersions of BFMT in PVP K 25, 5 ml of aqueous buffer solutions were added to the solution of BFMT and PVP K 25 (4.75 g total mass) in a mixture of methanol and water ($80 + 20$, v + v) before removing the solvent either by evaporation or freeze drying. The amounts of buffer

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salts added were 0.247 g citric acid and 0.038 g Na_2HPO_4 for pH 2.1, and 0.87 g KH_2PO_4 and 0.163 g Na_2HPO_4 for pH 7.0, respectively. To stress the samples (Table 1), relative humidities (RH) were obtained using saturated salt solutions as described by Nyqvist [2], in hygrostatic chambers. The compounds used were P_2O_5 (0%), $MgBr_2$ (30%), NaBr (53%) and NaCl (75%). These hygrostats were kept in incubators (B 5050 E, Heraeus, Hannover, Germany) at constant temperature. The actual temperatures were controlled every 3 days.

The samples of factorial design experiments were taken after 50 days (BFMT/PVP K 25) and 34 days (BFMT/PEG 6000, BFMT/Poloxamer 188), respectively.

For the studies of the kinetics of formation of associates in solid dispersions in PEG 6000 and in Poloxamer 188, HPLC was performed as previously reported by Frontini and Mielck [3]. Two different samples of the same solid dispersions were analysed in triplicate.

The statistical evaluation of the factorial design studies was performed as reported by Retzlaff et al. [4] using the software Statgraphics (Statistical Graphics, Rockville, MD, USA). Since two different samples were analysed at the same level, the variance of the responses could be well determined using the range R of the two samples according to Eq. (1) (with 2^n degrees of freedom)

$$s_0^2 = \frac{1}{2^n + 1} \sum_{1}^{2^n} R_i^2 \qquad (1)$$

where s_0 is variance of the response, n is number of the factors, R is range of the response between samples at the same level. The critical value of significance E_{crit} for an effect was calculated for $\alpha = 0.01$ according to:

$$E_{\mathrm{crit}} = \sqrt{\frac{F_{(0.01;\,1;\,2^n)} * s_0^2}{2^n + 1}} \qquad (2)$$

3. Results and discussion

Since during the stress time, a degradation of BFMT should be expected, experiments were performed at 0% RH to minimize hydrolysis of BFMT using solid dispersions of BFMT and of its degradation products. This confirmed that no differences were detectable in the formation of associates between BFMT and its degradation products. It is therefore reasonable to quantify the associate from the difference of the molar sum of the detected compounds and the initial amount of BFMT.

Table 2
Factorial design analysis of the formation of associates in solid dispersions of BFMT in PVP K 25

Factor	Effect [mol%]	Interaction	Effect [mol%]
%	−6.6[a]	%↔Man	0.1
Man	0.9	%↔pH	−1.9[a]
pH	1.5[a]	%↔RH	−3.2[a]
RH	7.3[a]	%↔T	−4.5[a]
T	7.8[a]	Man↔pH	−0.5
		Man↔RH	0.5
		Man↔T	0.7
		pH↔RH	1.0[a]
		pH↔T	0.7
		RH↔T	4.8[a]

The effects and interactions are expressed in mol%.
[a] Significant ($E_{\mathrm{crit}} = 0.95$ mol% for $\alpha = 0.01$).

The first factorial design experiment was performed using BFMT/PVP K 25 solid dispersions to test a large number of factors as summarized in Table 1.

Since the value of this series of studies lies in the accumulation of experience on the stability of solid dispersions of sparingly soluble drugs in hydrophilic polymers, the ratio of the amount of associate of BFMT and polymer to the amount of BFMT before stressing is the best response for factorial design analysis. Nevertheless, two factors are critical: first, a differentiation of associates of BFMT and polymer from associates of its degradation products and the polymer is not possible, and second, although the amount of 5% (w/w) of buffer should be enough to set the pH [5], Carstensen and Wan Po [6] emphasized that in solid dispersions the pH will not be the same as in the aqueous buffer solution. However, we assume the difference between the two pH values within the disper-

Table 1
Levels of the factorial design for the investigation of the formation of associates in solid dispersions of BFMT in PVP K 25

Factor	−	+
Percent of BFMT (w/w), (%)	3%	20%
Process of manufacture (Man)	Freeze drying	Evaporation
pH	2.1	7.0
Relative humidity (RH)	$MgBr_2 \simeq 30\%$	$NaBr_2 \simeq 53\%$
Temperature (T)	30.2 ± 0.7°C	60.2 ± 0.7°C

Abbreviations of the factors are put in brackets.

Table 3
Levels of the factorial design for the investigation of the formation of associates in solid dispersions of BFMT in PEG 6000 and in Poloxamer 188

Factor	−	+
Polymer (Pol)	Poloxamer 188	PEG 6 000
Percent of BFMT (w/w), (%)	3%	10%
Relative humidity (RH)	$MgBr_2 \simeq 30\%$	$NaBr_2 \simeq 53\%$
Temperature (T)	30.2 ± 0.4°C	48.2 ± 0.1°C

Abbreviations of the factors are put in brackets.

Table 4
Factorial design analysis of the formation of associates in solid dispersions of BFMT in PEG 6000 and in Poloxamer 188

Factor	Effect [mol%]	Interaction	Effect [mol%]
%	−5.2[a]	%↔RH	−1.5[a]
RH	1.6[a]	%↔T	−2.7[a]
T	4.8[a]	%↔Pol	2.0[a]
Pol	−6.1[a]	RH↔T	1.9[a]
		RH↔Pol	−2.6[a]
		T↔Pol	−2.7[a]

The effects and interactions are expressed in mole %.
[a] Significant ($E_{crit} = 0.7$ mol% for $\alpha = 0.01$).

sions to be close to that for the respective buffer solutions, so that the effect of pH in a factorial design may well be assigned to this difference.

Table 2 summarizes the results of the factorial design analysis.

The content of BFMT in the solid dispersion has a highly significant effect as expected. Since the absolute amount of BFMT may be decisive for the discussion of this effect, a separate experiment was performed using solid dispersions of the main degradation product TFSA in PVP K 25 which will be discussed below.

The process of manufacturing of the solid dispersions does not have any significant effect.

The effect of pH is positive but not very pronounced in accordance with the results of Ågren and Bäck [7] for human albumin. Possibly the undissociated species of BFMT forms a more stable 'complex'. The negative influence of the interaction %↔pH may confirm this fact because a larger amount of BFMT will decrease the dissociation of BFMT and consequently the effect of pH. The positive but hardly significant influence of the interaction pH↔RH may be interpreted on the other hand as caused by increased dissociation.

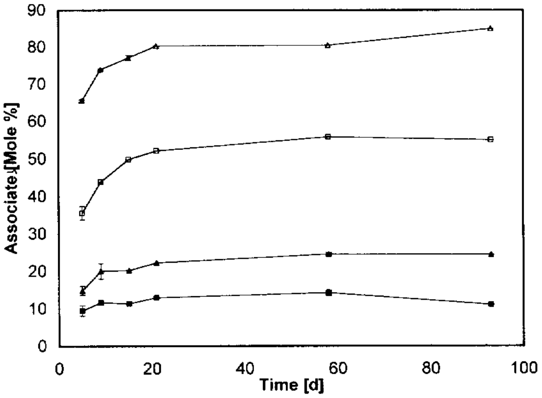

Fig. 1. Effect of TFSA concentration (w/w) on the formation of associates in solid dispersions of TFSA in PVP K 25, stressed at 60°C and 50% RH. The amount of associates is expressed in mol% of the initial amount of TFSA. Means of triplicate analyses of two different samples and range of single values. (△) 1%, (□) 3%, (▲) 10%, (■) 15%.

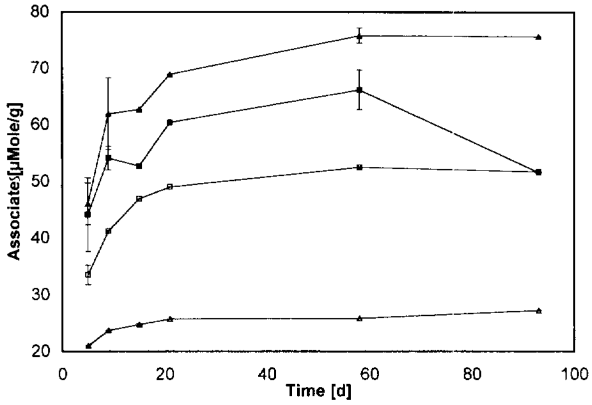

Fig. 2. Effect of TFSA concentration on the formation of absolute amount of associates per mass of solid dispersion of TFSA in PVP K 25, stressed at 60°C and 50% RH. Means of triplicate analyses of two different samples and range of single values. (△) 1%, (□) 3%, (▲) 10%, (■) 15%.

Relative humidity and temperature have the most important and positive effects. Oksanen and Zografi [8] showed that water is an effective plasticizer for PVP. Since the interaction RH↔T has a large positive influence, we may postulate that the glass transition temperature, T_g, and the melting point are the most important factors influencing the formation of associates. A decrease of T_g promotes the formation of associates. On the other hand, the interactions %↔T and %↔RH have a negative influence. This may be explained by the fact that hydrophobic species like BFMT promote the formation of clusters in water [9] and consequently reduce its function as plasticizer.

In a second factorial design, the effect of the polymers PEG 6000 and Poloxamer 188 was investigated together with those factors from the first design, which were highly significant. Thus, the effects of process of manufacture and of the pH were not studied further.

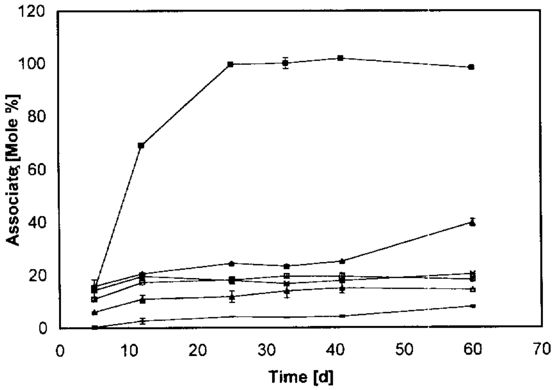

Fig. 3. Effect of temperature on the formation of associates in solid dispersions of BFMT (3% w/w) in PEG 6000, stressed at 0% RH and different temperatures. Means of triplicate analyses of two different samples and range of single values. (——) 21°, (△) 35°, (□) 45°, (☆) 50°, (▲) 55°, (■) 60°.

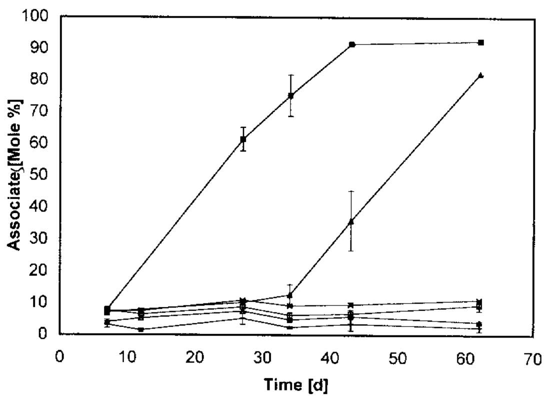

Fig. 4. Effect of temperature on the formation of associates in solid dispersions of BFMT (3% w/w) in Poloxamer 188, stressed at 0% RH and different temperatures. Means of triplicate analyses of two different samples and range of single values. (——) 21°, (△) 35°, (□) 40°, (☆) 45°, (▲) 50°, (■) 55°.

The design is given in Table 3 and the results are summarized in Table 4.

The results confirmed the first experiment. In addition, the effect of the type of polymer seems to be highly significant. The formation of associates is promoted in Poloxamer 188 possibly because this polymer has quasi-tenside properties. The hypothesis of formation of clusters elucidates the positive effect of the interaction %↔Pol since PEG 6000 is not able to reduce clusters like the quasi-tenside Poloxamer 188. The negative effect of the interactions RH↔Pol and T↔Pol may be interpreted as a verification of the relevance of the T_g and the melting point as a polymer specific property, since the T_g and the melting point of Poloxamer 188 are lower than those of PEG 6000.

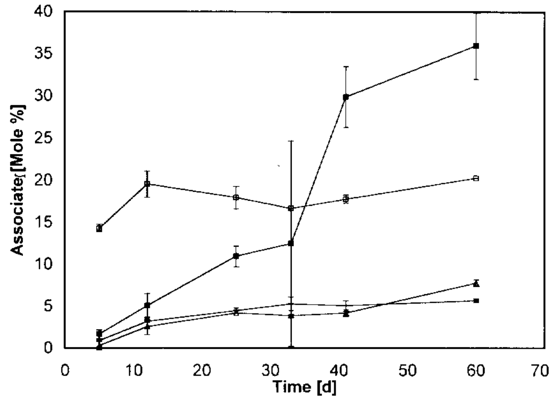

Fig. 5. Effect of humidity, 75% RH in comparison with 0% RH, on the formation of associates in solid dispersions of BFMT (3% w/w) in PEG 6000, stressed at 21 and 50°C. Means of triplicate analyses of two different samples and range of single values. (■) 50°C, 75% RH; (□) 50°C, 0% RH; (——) 21°C, 75% RH; (△) 21°C, 0% RH.

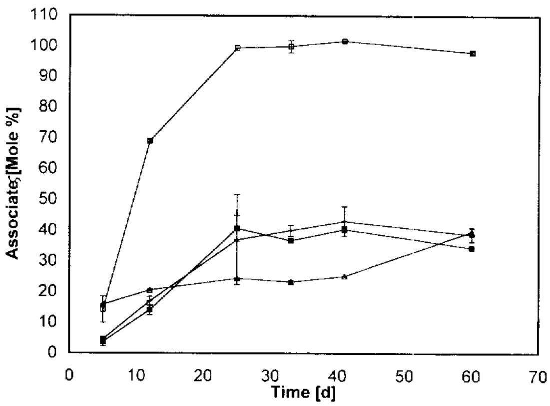

Fig. 6. Effect of humidity, 75% RH in comparison with 0% RH, on the formation of associates in solid dispersions of BFMT (3% w/w) in Poloxamer 188, stressed at 55 and 60°C. Means of triplicate analyses of two different samples and range of single values. (■) 60°C, 75% RH; (□) 60°C, 0% RH; (——) 55°C, 75% RH; (△) 55°C, 0% RH.

3.1. Concentration of guest

The highly significant effect of the concentration of BFMT in the solid dispersions was studied in more detail in the system TFSA/PVP K 25 since high chemical stability of the guest is required to perform a kinetic study with this response. We stressed these dispersions at 50% RH and 60°C to obtain a large amount of associates and thereby to minimize the experimental error. Four different percentiles of the guest, high temperature close to the melting point of the solid dispersions, of TFSA, namely 1, 3, 10 and 15% (w/w), were investigated during 93 days and quantified in mol% as well as in absolute amount of associates (μmol*g^{-1}). (Figs. 1 and 2).

The formation of the associates is a slow process and a steady state is reached after about 25 days. Furthermore, the results confirm that a high concentration of guest inhibits the association. On the other hand, Fig. 2 highlights that the absolute quantity of associates formed approaches a maximum since the amount formed in 10% solid dispersions is always higher then that in 15% solid dispersions. It is reasonable to suppose that not more then 75 μmol·g^{-1} are formed, i.e. 24 mg·g^{-1}. Solid dispersions stressed under other conditions, namely 30% RH/30°C, 30% RH/60°C and 50% RH/30°C, confirmed these results (results not shown).

3.2. Temperature

The influence of the temperature was studied carefully in 3% (w/w) solid dispersions of BFMT in PEG 6000 and of BFMT in Poloxamer 188, which were stressed at 0% RH (Figs. 3 and 4).

Obviously, a critical temperature between 55 and 60°C for PEG 6000 and between 50 and 55°C for Poloxamer 188 is needed for the formation of high amounts of associates. Since the melting point of PEG 6000 is about 59°C and that of Poloxamer 188 is about 54°C it is evident that the formation of associates is highly favoured in the liquid state. The observed increase of associates (Fig. 4) after about 35 days at 55°C for PEG and at 50°C for Poloxamer 188, respectively, might be caused by phenylacetaldehyde which is a product of hydrolysis of BFMT and should be able to act as a plasticizer and thus decrease the melting point. Hydrolysis of BFMT was observed after about 25 days inspite of the 0% RH in the surrounding atmosphere. Data on the hydrolysis will be reported in Part III [10].

3.3. Relative humidity

The influence of the humidity is elucidated by the comparison of dispersions stressed at 0% and 75% RH, respectively, below and close to the critical temperature as shown in Fig. 5 for the dispersion of BFMT in PEG 6000.

No effect is observed at 21°C, but close to the melting point of the polymer, water as a plasticizer is able to decrease the melting point and thereby promote the formation of associates. This correlates with the liquifying of the polymer, which was observed macroscopically under these conditions. Above the melting point of PEG 6000, high humidity has an opposite effect as shown in Fig. 6.

The formation of associates seems to be correlated to a narrow range of viscosity, i.e. low as well as high mobility of the molecules will have a negative effect. Similar effects were observed for the dispersions Poloxamer 188, but as expected between 5 and 10°C below the temperatures characteristic for the dispersions in PEG 6000 (results not shown). Since at 75% RH above 50 and 40°C, respectively, for dispersions in PEG and in Poloxamer 188 the samples were liquid we can conclude that the formation of associates is promoted by temperatures close to the melting point of the solid dispersions but not in the less viscous colloidal solutions, which will exist at higher temperatures.

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