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Screening chemical and physical stability of drug substances*

MATS BERGLUND, KATARINA BYSTRÖM and BJÖRN PERSSON†

AB Draco (Subsidiary to AB Astra), Research and Development Department, Analytical Chemistry, P.O. Box 34, S-221 00 Lund, Sweden

Abstract: General multistep methods are described for the screening of the chemical and the physical stability and/or reactivity of new drug candidates. The chemical reactivity is studied in aqueous buffer solutions; the rate of degradation is measured as a function of pH. The products of the reaction mixture are evaluated at selected pH-values as a function of time and key reaction products are identified. Strategies for testing degradation products in stability studies are discussed. The purpose of the physical reactivity test is to obtain information on the existing solid-state form in relation to the thermodynamically stable form. A method for finding the stable form is described. When polymorphism is observed a search for additional polymorphs is performed and the different solid phases are characterized. Special tests are described for hydrates and anhydrous forms.

Keywords: *Chemical stability; degradation products; physical stability; polymorphism; pre-formulation study.*

Introduction

Basic information about the stability (or conversely the reactivity) of the active substance is needed at an early stage in the development of a new drug. A broad base of knowledge makes it possible to avoid problems and pitfalls in the development work. The need for adequate information is especially pronounced in the case of an unstable drug molecule, when the information is essential in the development of the method of synthesis and in the formulation work.

A special need associated with chemical reactivity is the development of stability-indicating analytical methods for the determination of degradation products in the active substance and its dosage forms. A prerequisite for rational method development is knowledge about the identity of actual and/or conceivable degradation products.

Physically reactive drug substances, i.e. substances in an unstable or metastable form, or amorphous solids undergo phase transitions to the thermodynamically stable form. Such transitions may induce changes in the technical and biopharmaceutical properties of powders and solid dosage forms [1]. These types of problem can be eliminated if the final purification step can be modified to give the stable form. A similar situation exists for substances crystallizing as solvates and/or hydrates. Drug substances in the form of a salt have different physical and chemical properties depending on the counter-ion. Early screening studies give an opportunity of detecting potential problems which may be solved simply by a change of counter-ion.

Generally, a chemical stability study is included in the preformulation programme for a new drug candidate [2, 3]. Initial physical tests used to be limited to a study of hygroscopicity. The stability of the solid-state form is usually investigated at a later stage. The purpose of the present report is to describe general methods for early screening of chemical and physical reactivity. The development of the suggested methods is based on experience of a number of different types of drug substances used in respiratory medicine. Especially, the increasing use of dry powders for inhalation has emphasized the need for adequate testing of physical reactivity.

Experimental

A variety of liquid chromatographic methods has been used in the chemical degrad-

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†Author to whom correspondence should be addressed.

ation studies. Qualitative chemical analysis has been performed on-line by liquid chromatography coupled with thermospray mass spectrometry (LC/TSP-MS; VG Trio 2) or with multiwavelength spectrophotometry (LC/UV-DAD; HP 1040A diode array). Solid-state forms have been characterized by differential scanning calorimetry (DSC; Perkin–Elmer DSC7), thermal gravimetric analysis (TGA; Perkin–Elmer TGA7) and X-ray powder diffraction (Scintag XDS 2000).

Results and Discussion

Chemical reactivity

A multistep scheme for studying the chemical degradation of a drug substance in aqueous buffer solutions was developed (Fig. 1). Substances with poor solubility in water were handled by the use of mixtures of water and organic solvents. Extremely lipophilic compounds did not fit into the scheme; probably, water-based reactions are of limited interest for this class of compound. For stable molecules it was sufficient to perform the initial screening step, whereas reactive substances required extensive studies through all the steps.

In the first step of the scheme, experimental conditions were established under which it was possible to measure the degradation rate on a reasonable time scale. Elevated temperatures were used, but too high temperatures (>60°C) may introduce new types of reactions like eliminations and rearrangements that do not reflect the situation at room temperature. In the case of metal-ion catalysed oxidations a small, constant amount of a cupric salt was added. Preferably, a factorial design could be used for the screening step.

In the second step the rate of disappearance of the active substance was studied as a function of pH. The pseudo-first-order rate constant (k) for the extrapolated initial rate was evaluated from the measurement of the active substance as a function of time in different buffer solutions. The pH profile defined as the plot of log k versus pH provided information on, for example, acid and base catalysis in the degradation reaction.

The purpose of the third step was to gain information on how degradation products and intermediates varied as a function of time in some buffer solutions selected on basis of the pH-profile. From these product profiles it was possible to find out the major degradation products (key reaction products) to give information about the degree of degradation of the active substance.

The last step of the scheme involved identification of the key reaction products. The combined results from LC/TSP-MS and LC/UV-DAD often gave enough information to allow a qualified assumption of the molecular structure, which was confirmed by comparison with an authentic sample. In more complicated cases preparative isolation was required.

Taken together, the results from the four steps made it possible to arrive at the reaction mechanisms for the degradation. The relative importance of different reactions, e.g. hydrolysis and oxidation, could be distinguished. The pH-region for the presence of key reaction products was evaluated and used for the postulation of conceivable degradation products in the active substance and in formulations. The source of undesirable degradation in a dosage form could be identified.

An overall view of the chemical stability of a drug candidate is obtained by combining the

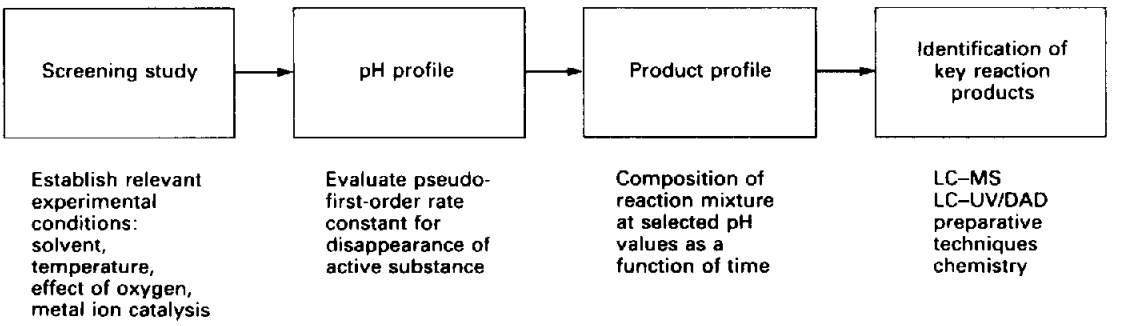

Figure 1
Scheme for chemical degradation studies in aqueous buffer solutions.

information from different stability tests such as a chemical reactivity test, an accelerated stability test of the pure substance and a photochemical reactivity test.

Degradation products in stability studies

Stability studies of drug products must include tests for degradation products. Depending on whether or not the drug product is unstable and whether degradation products actually have been found, some different situations may arise. In all these situations the results from the chemical reactivity test will form a rational basis for the design and the evaluation of long-term stability tests. Two examples are given.

Bambuterol is a pro-drug for the treatment of asthma. The substance is the bis-dimethylcarbamate of terbutaline (Fig. 2a). Chemical reactivity studies revealed that bambuterol was stable in weakly acidic to neutral solutions and that the only key reaction product in acidic and alkaline solutions was the monocarbamate derivative. Thus the stability-indicating analytical method must be capable of separating and determining bambuterol and the monocarbamate derivative [4].

The xanthine derivative, enprofylline, in Fig. 2(b), was found to be perfectly stable in pure form and in aqueous solutions of pH 1–11. Thus, no key reaction product could be pointed out in this way. Since ring-closure

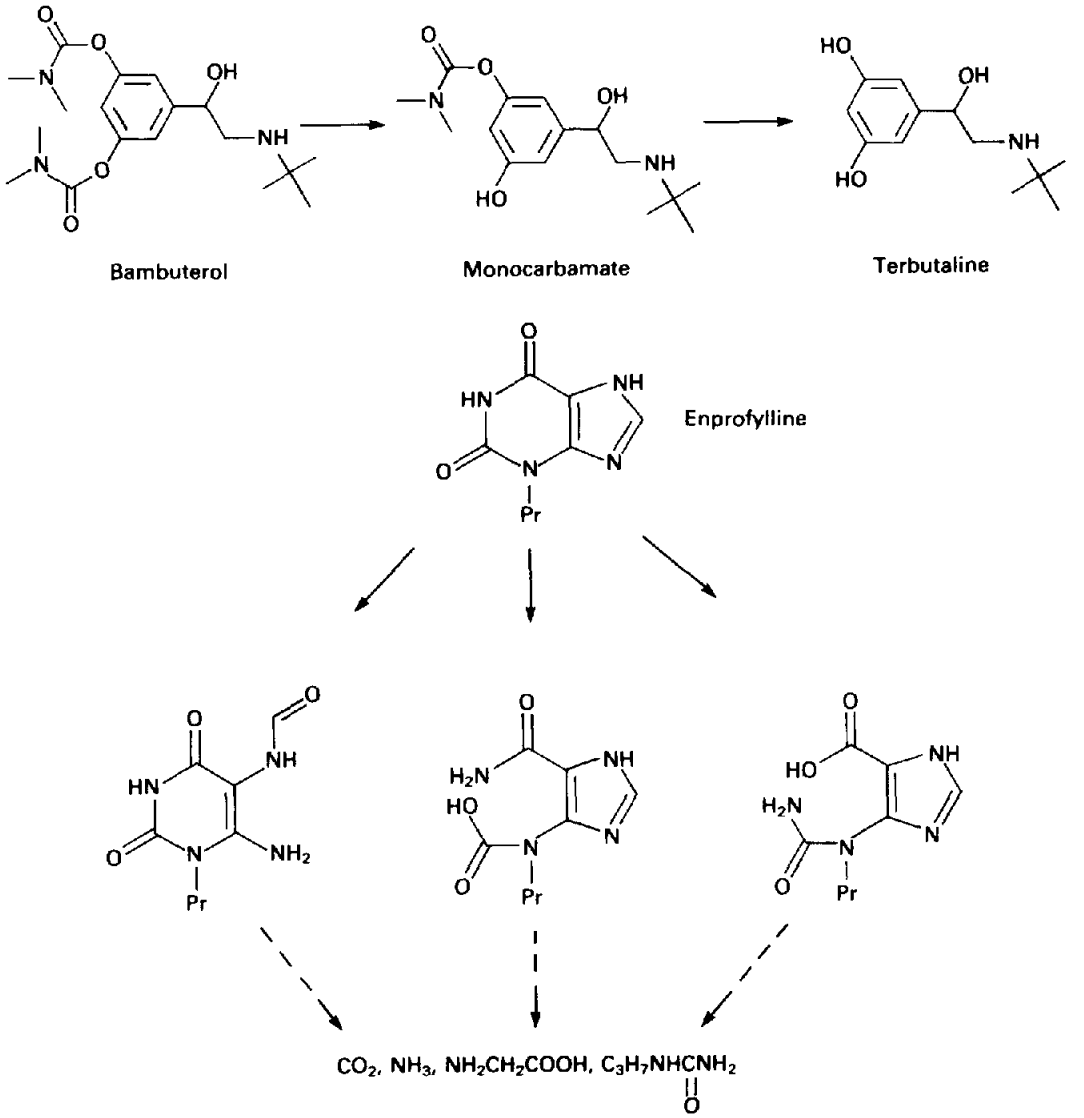

Figure 2
(a) Chemical degradation of bambuterol; one reaction pathway and one key reaction product (monocarbamate). (b) Chemical degradation of enprofylline; several reaction pathways without any key reaction products.

reactions and recrystallizations were conducted in strongly alkaline solutions with losses of material through hydrolysis, it was of interest to study the degradation in 1 M NaOH. The degradation proceeded all the way down to the basic building stones of the molecule such as glycine and propylurea. The product profiles showed very small concentrations of a large number of intermediates but no key reaction products could be found under these conditions either. Obviously, there is neither the possibility nor the need of designing a method for the determination of degradation products in formulations of enprofylline.

Physical reactivity

The scheme developed for the screening of solid-state properties and physical reactivity starts with a solid-phase analysis of all batches produced by the actual method of synthesis (Fig. 3). The three techniques used in this study gave information on changes of mass (TGA), energy transfer (DSC) and structure (X-ray powder diffraction), which taken together complemented each other. Mixtures of solid phases were characterized by X-ray diffraction, although peak overlap did cause some problems. Amorphous solids were recognized from the lack of distinct peaks in the X-ray diffractograms.

To allow predictions about physical reactivity and phase transitions the second step involved the establishment of the thermodynamically stable solid phase. This was done by suspending the solid substance in a solvent where the solubility was not too small (~1 mg ml^{-1}). Equilibration in this way produced the stable solid phase. An exception would be where a solvate was formed with the solvent used.

The method is illustrated by an application for two polymorphs of bambuterol hydrochloride (Fig. 4). Form I was recrystallized from acetone and form II from acetone–water. From the solubilities in water, it was deduced that form II ought to be the stable form at room temperature. However, the melting points showed that form I is the stable form at high temperatures. Both forms were equilibrated in the two solvent systems used for crystallization. In all experiments, only form II remained at equilibrium, showing that this polymorph is thermodynamically stable.

When polymorphism was observed among existing batches, a general search for additional polymorphs was undertaken in the third step of the scheme. This was done by recrystallization from a number of selected solvents covering a broad range of polarities, dielectric constants and hydrogen-bonding properties. Polymorphic forms and/or amorphous solids were also formed in certain pharmaceutical operations like spray drying and lyophilization.

In the fourth step, the solid phases were characterized with respect to properties of pharmaceutical–technical interest like solubility, hygroscopicity and crystal habit. The kinetics of transition to the thermodynamically stable form, i.e. the physical reactivity in a

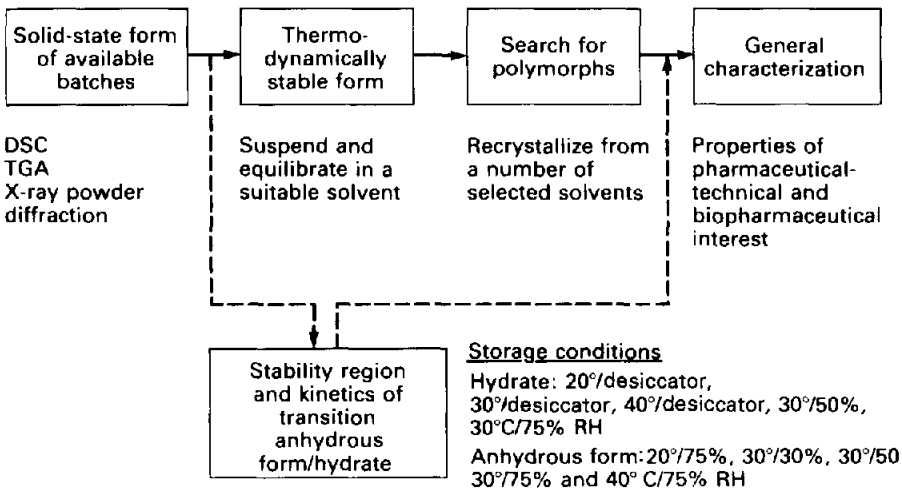

Figure 3
Scheme for studies of solid-state properties and physical reactivity.

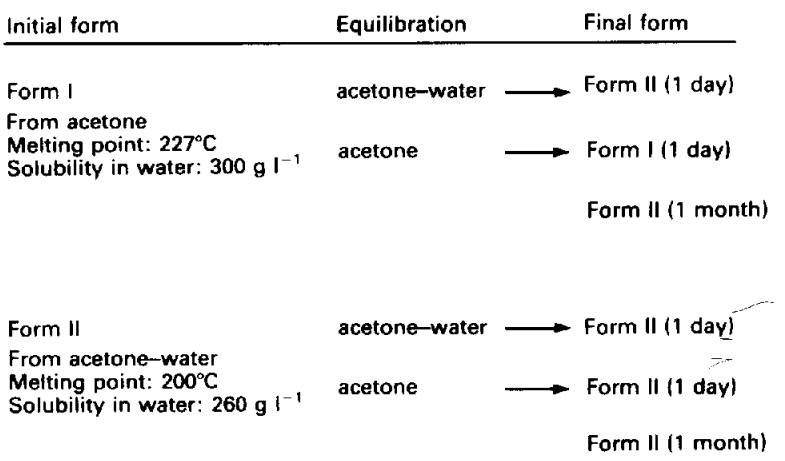

Figure 4
Equilibrium experiment with two polymorphs of bambuterol hydrochloride.

proper sense, was investigated. Metastable forms and even amorphous solids may stay unchanged for years.

The physical thermodynamic stability of hydrates and anhydrous forms is dependent on relative humidity and temperature. To find out the stability region and the rate of giving off or taking up water, the substance was stored under the conditions given in Fig. 3. Samples were analysed after 1, 3 and 6 months, etc. depending on the rate of polymorphic transition. In the final choice between a hydrate and an anhydrous form the stability region and the rate of polymorphic transition was considered in relation to normal storage conditions and normal handling of the active substance and to the formulated product.

The purpose of screening the solid-state properties of a drug candidate was to obtain information on the existing solid-state form in relation to the thermodynamically stable form. At a later stage of the drug development a comprehensive study of the solid-state properties will be required. The main focus at that stage will be polymorphism in relation to biopharmaceutical properties.

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