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The automation of dissolution testing of solid oral dosage forms*

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Abstract: Dissolution testing of solid oral dosage forms plays a very important part both in the development of new products and in quality control. A fully automated system for dissolution testing known as AUTO DISS® is presented and its components are described. On-line determination of active ingredient concentration is possible with the aid of an integrated automatic sampler in combination with various measuring instruments (UV–vis spectrometry, liquid chromatography and flow injection analysis). The suitability of the system is demonstrated by determination of the dissolution of brotizolam from tablets by FIA and of bepafant from capsules by diode-array spectroscopy.

Keywords: *AUTO DISS®; automated dissolution-rate system; paddle method; UV–vis spectrophotometry; diode-array spectroscopy; HPLC; FIA.*

Introduction

One of the most frequently performed tests in the pharmaceutical laboratory is dissolution. Tests of dissolution are important in: the development of new products, especially in optimizing the bioavailability of a drug substance; stability testing, i.e. in detecting changes in drug release under certain storage conditions; and quality control testing, i.e. in establishing lot-to-lot equivalence of formulations.

The most widely used dissolution technique is the paddle method, proposed by Levy and Hayes in 1960 [1] and modified by Poole [2]. This test is essential in almost all important pharmacopoeias. Data for registration at both the CTA (Clinical Trial Application) and NDA (New Drug Application) stage have to be presented in the submitted documentation. The manual procedure of dissolution testing is expensive and time consuming. In the development phase of stability studies, dissolution testing accounts for about 30% of all stability tests.

Soon after dissolution testing was introduced, researchers began suggesting ways of automating sampling and determination of the dissolved drug [3–9]. Sampling techniques are now highly advanced and complete installations are offered by vendors. Using these approaches to the automation of dissolution, it was possible to reduce the time required for the test. This automation stage, however, is restricted to the testing of one batch. The realization of a fully automated system which analyses more than one batch requires that additional activities like sampling, dropping tablets into vessels, draining the dissolution medium, and cleaning and drying of vessels should be carried out mechanically.

Dissolution testing can be automated by using laboratory robots [10–12]. These systems are now sophisticated enough for more complicated operating steps of the kind required in dissolution testing to be carried out.

One serious disadvantage of robotic automation, however, is that all the working steps have to be carried out sequentially, which results in long processing times. In particular, dissolution profiles with short sampling intervals present difficulties. Furthermore, in many cases robotic systems are not very suitable for processing smaller series of test samples for which a frequent change of method is required, because the flexibility of such systems is limited.

The present paper describes a more advanced system which was recently published [13, 14]. This system differs from laboratory

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robot-assisted systems in that it can carry out all operating steps synchronously. The apparatus (AUTO DISS®) can also be used for fully automated testing of sustained-release dosage forms. This requires that a change of buffer from simulated gastric fluid to simulated intestinal fluid according to USP can be effected mechanically.

Automated Dissolution Testing with the AUTO DISS System

Concept

The concept of automated dissolution testing extending from filling of dissolution vessels to evaluation of analytical data is presented schematically in Fig. 1.

After the parameters for the selected method are entered, the system is started and the following operations are carried out automatically in a single cycle: filling vessels with dissolution medium; heating the medium to the required temperature; dropping dosage forms into vessels; adjustment of paddle speed; sampling at pre-set times and transfer of solution to analytical instruments via the autosampler; change of dissolution medium from gastric fluid to intestinal fluid for sustained-release dosage forms by adding buffer solutions; and draining, rinsing and drying of dissolution vessels. On completion of each separation step, the next cycle starts automatically in accordance with the preselected program.

Data collection and reduction, statistical calculation, report generation and presentation of data can be effected and printouts supplied via the PC-based controller after the analytical determination. Method and data management is carried out on a database system.

Components of the AUTO DISS system (Pharma Test GmbH, Hainburg, Germany)

The system consists of various elements (Fig. 2). The paddle apparatus comprises six vessels for tests and one vessel for blank. The vessels and paddles are modified such that draining of the medium, rinsing and drying can be executed automatically. Magazines are provided for each vessel (under nitrogen) for the storage of tablets or capsules for up to 20 batches. The automatic system is equipped with syringes and valves for filling the vessels with dissolution medium, changing the pH of the medium (important for sustained-release dosage forms) and transferring the samples to an autosampler. A Gilson 232/401 (Gilson France, Villiers le Bel) autosampler for 360 samples is used; this is equipped with two Rheodyne valves for transferring the test and standard solutions to HPLC, FIA, spectrophotometric or other instruments. A micro-

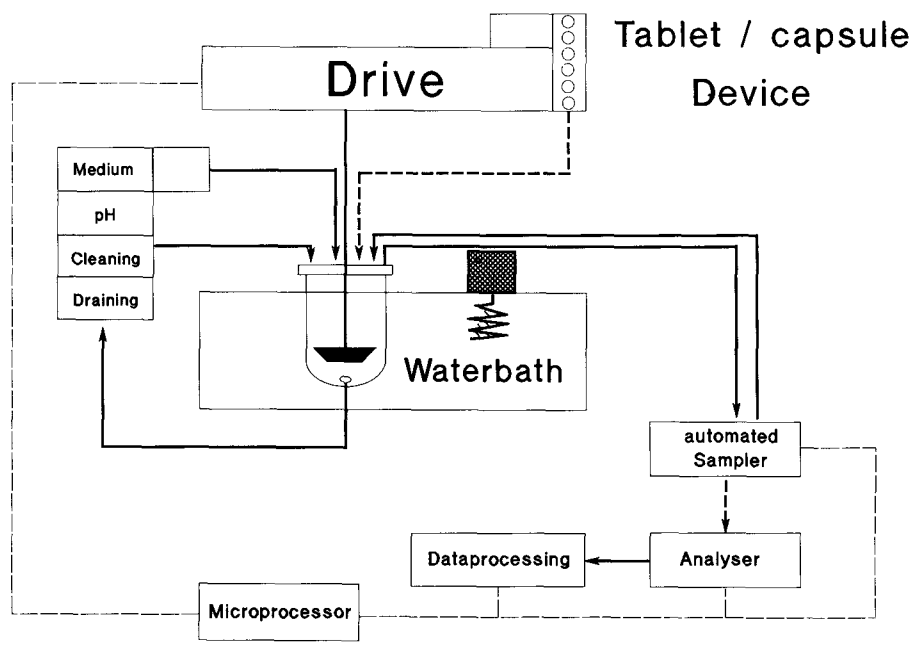

Figure 1
Concept of automated dissolution testing.

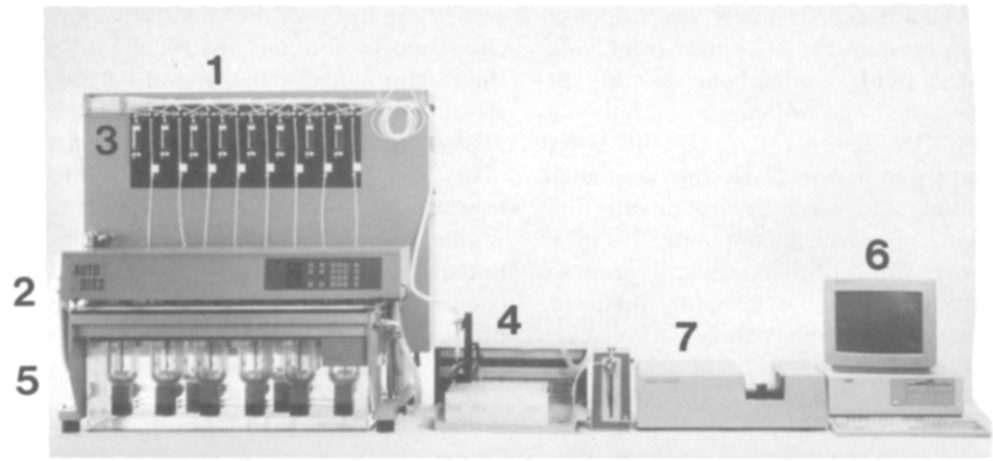

Figure 2
The components of the AUTO DISS system. 1, Dissolution tester; 2, automatic magazine; 3, automatic filling system; 4, sampler system; 5, draining system; 6, controller unit; 7, UV–vis spectrophotometer.

processor (MS DOS, IBM-compatible) is used to control the system and document the test parameters. Standard software is employed for entering and storing methods and data for evaluation and archiving.

Operating procedure. The operating procedure for one cycle, in which all the steps from filling the vessels with dissolution medium to cleaning and drying of the vessels are controlled automatically via a microcomputer, is shown in Fig. 3. As many as 20 cycles can be processed automatically in succession, with up to six individual tablets being tested per cycle. Before serial testing is started parameterization is first performed via the dialogue program. The following variables are established: number of cycles (max n = 20, i.e. 20 × 6 tablets); volume of dissolution medium (250–1000 ml); temperature of dissolution medium (RT–45°C); paddle speed (25–250 rpm); test solutions–volume (1–50 ml), number and time of samplings; rinsing times and rinsing volumes for sampling tubes; buffer solutions for pH change — volume dosing depending on type of buffer and required pH; sample coding in autosampler system for identification for measurement and documentation of samples; cleaning steps for vessels and paddles; and rinsing of syringe station and all tubes.

After the parameters have been entered, the dissolution vessels are filled simultaneously with the dissolution medium from a storage vessel by means of a syringe system. After a constant temperature has been reached, the

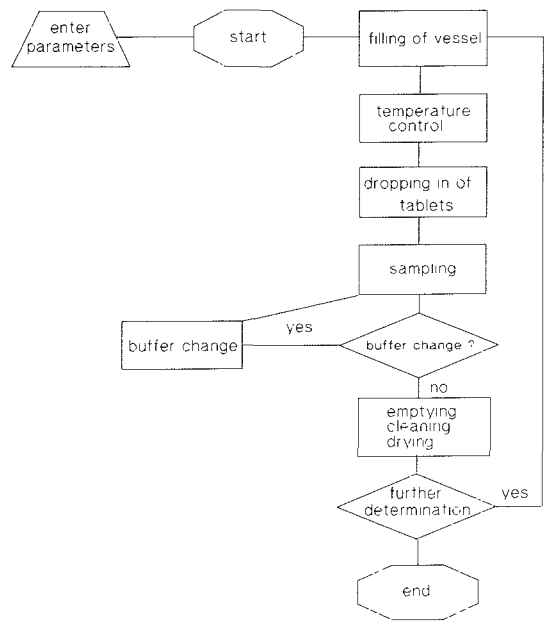

Figure 3
Program flow diagram of operating steps within a cycle.

microcomputer activates the drive motor for the sampling magazine. When the motor has moved the sampling magazine one step hole onward, the microcomputer switches off the motor. The samples to be tested fall simultaneously through specially positioned apertures into the individual dissolution vessels. The paddles are then set in motion. The solutions to be measured are transferred according to a preselected program via the dispenser station directly to a Gilson 232 autosampler or optionally to the multicell transport system of a spectrophotometer. For

sustained-release preparations, the dispenser station also changes the pH buffer from simulated gastric fluid simultaneously in all dissolution vessels. In accordance with Method A of USP XXII, 250-ml quantities of buffer solution are pumped from storage containers through integrated valve circuits directly into the receiving vessels each containing 750 ml of 0.1 M hydrochloric acid. Automatic drainage of the dissolution medium, cleaning and drying of the vessels and the paddles have been described in detail in ref. 14. After this cleaning step, the first cycle is completed and the dissolution vessels can be filled again with dissolution medium.

The functioning and validation of the AUTO DISS system and its suitability both for quality control and research and development applications have been described [13].

Methods of analysis

The release of an active ingredient from pharmaceutical formulations is generally determined using analytical methods that are readily adaptable to release testing apparatus and which themselves are highly automated.

Methods that meet these requirements are UV–vis spectroscopy, photodiode array spectroscopy, high-performance liquid chromatography (HPLC) and flow injection analysis (FIA).

All these methods can be used in combination with the AUTO DISS system, the release solutions first being transferred to the Gilson 232 autosampler. This system serves not only as a fraction collector but also as a sample preparation system for dilution, concentration, derivatization and transfer of the test solutions to the on-line analyzer. Altogether as many as 360 samples can be processed.

UV–vis spectrometry [3]. The use of UV–vis spectrophotometry for the determination of the release of an active ingredient is adequately documented [3]. Combination with the AUTO DISS system can be effected in two different ways. In the first modality the spectrophotometer equipped with a flow cell is supplied with test solution via the Gilson 232. In the second configuration, the spectrophotometer is connected directly to the AUTO DISS system. In the latter case, UV–vis spectrophotometers equipped with several flow cells and an automatic multicell transport system are used. The flow cells are connected to the dissolution vessels via Teflon tubes and the solutions are circulated by a pump. To record the spectra, the flow is halted. This type of system has the advantage of providing easy, fast and low-cost analysis of the released active ingredient.

UV–vis spectroscopy is generally unsuitable, however, if several active ingredients with similar spectral characteristics are to be determined in multicomponent products or if matrix components interfere with the determination of the active ingredient because they absorb in the same range of wavelengths.

Photodiode array spectroscopy [15, 16]. In contrast to conventional spectrophotometers, diode-array instruments measure the absorbance at all wavelengths simultaneously. This allows special mathematical processes such as multicomponent analysis to be used. A further advantage is that reference spectra can be stored so that standards only need to be measured occasionally for control purposes. Multicomponent analysis is able to eliminate unwanted sources of absorption in dissolution determinations. In dissolution tests on capsule formulations, unwanted absorption is contributed both by the formulation excipients and by the gelatin shell, which dissolves completely in the dissolution medium. Absorption due to the active ingredient and the placebo (the formulation excipients plus the gelatin shell) is shown in Fig. 4 for bepafant capsules (5.0 mg) [15].

Dissolution testing was carried out by the paddle method [2]. Details of the method are given under 'Apparatus 2' in section 711 of USP. The reproducibility of the method was tested using the filtered solution obtained at the end of the dissolution test. The solution was determined 10 times. Linearity and accuracy were tested at concentrations between 50 and 120% of the working concentration for the method. At each concentration level, solutions were tested with and without an added placebo capsule (comprising the gelatin shell and the formulation excipients). All solutions were filtered prior to measurement.

The test conditions were: dissolution apparatus, AUTO DISS; dissolution medium, water (500 ml); method, paddle method; operating time, 30 min; paddle speed, 50 rpm; temperature, 37 ± 0.5°C; instrument, Hewlett-Packard 8452 A; and evaluation: Quant II (software for multicomponent analysis).

The method conditions were: wavelength

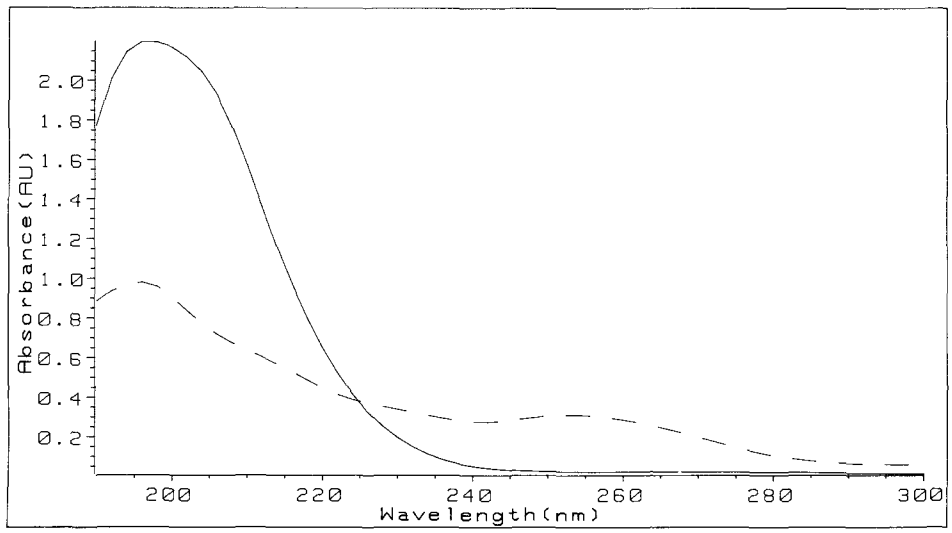

Figure 4
Spectra of bepafant and the placebo capsule (formulation excipients and gelatin capsule shell). -----, 5.0 mg bepafant in 500 ml of water; ————, placebo capsule.

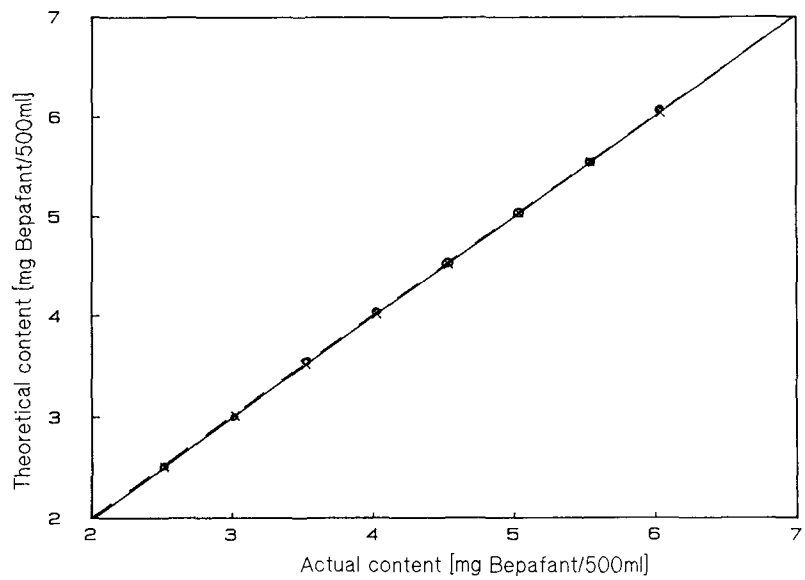

Figure 5
Bepafant capsules 5.0 mg: linearity, accuracy and excipient interference. Range: 50–120% of working concentration (2.5–6.0 mg bepafant in 500 ml water). □, Without placebo capsule; ×, with placebo capsule.

range, 276–286 nm; data type, first derivative with three-point smoothing; analytical functions (described to reduce instrument noise): spectrum — average value of 400–450 nm, and signal averaging time, 0.5 s.

The reproducibility determined as the RSD was 0.14% (n = 10). Linearity and accuracy were shown to be satisfactory; the average bias was 0.16% (see Fig. 5).

The investigations show that diode-array spectroscopy can be successfully used to perform release tests on pharmaceutical dosage forms. The method is very fast (60 samples h^{-1}) and cost-effective (no consumption of reagents and materials); wherever possible, the method can be substituted for less cost-effective and more time-consuming methods such as chromatographic techniques [15].

High-performance liquid chromatography [6, 9, 17, 18]. HPLC systems are also readily adaptable to the AUTO DISS system, since

the Gilson 232 autosampler was originally developed for chromatographic use. LC is a more sensitive and specific technique than UV methods. Generally, LC is used to determine the dissolution of multicomponent pharmaceutical formulations and to analyse sustained-release products and low-dose formulations in which interference problems from excipients and coating materials can be more severe.

Fast and robust LC with short columns packed with 3-μm particles is preferred since analysis times have been cut from 20 min to about 5 min. The need for robust LC methods in dissolution testing is obvious because dissolution testing involves high-volume assays that require a high degree of accuracy. However, reproducibility problems and systematic errors can occur when test samples with complex matrices are under investigation [15]. Interference can be expected if the test sample contains macromolecular excipients such as polyvinylpyrrolidone or ethylcellulose. Gelatin, in particular, repeatedly causes serious interference problems in analytical procedures designed to determine dissolution rate in capsule formulations.

Chromatographic methods used in this application suffer from loss of reproducibility in serial analysis because protein constituents present in the gelatin absorb to the surface of the stationary phase and alter its polarity.

Flow injection analysis [19–21]. Combination of FIA with the AUTO DISS system differs from the HPLC variant only insofar as the solution to be measured is directly injected at a constant rate via the Rheodyne valve of the Gilson 232 autosampler into a transport or reagent flow. A chemical reaction can immediately occur in the reagent flow due to diffusion and convection and the resulting product can be recorded in the form of a peak on a flow detector connected to the mixing loop.

Determination of the release of the active ingredient from brotizolam in Lendormin tablets is described as an example [20]. The paddle method in the modification of Poole [2] is used to determine the release of brotizolam. Numerous samples are analysed in this *in vitro* test, as the specifications require that a population of not less than six individual samples are to be tested per batch.

Procedure: six Lendormin tablets (0.5 mg) were each incubated in 500 ml of water for 30 min at 37°C in the AUTO DISS system. Aliquots of the resulting solutions were then filtered and transferred synchronously to the Gilson 232 autosampler. Figure 6 presents a flow diagram for the determination of brotizolam.

The method conditions were: carrier flow, 0.2 M hydrochloric acid, degassed, 1.5 ml min^{-1}; injection volume, 100 μl equivalent to 100 ng of brotizolam; detection, excitation wavelength λ = 300 nm and emission wavelength λ = 480 nm; and cell volume, 12 μl.

The calibration curve was plotted in the concentration range 50–150 ng, the influence of the tablet excipients being checked over the entire measurement range. The following regression equations were calculated. Without excipient: $y = a + bx$; $a = -4566$; $b = 1283010$. With excipient: $y = a + bx$; $a = 13866$; $b = 1247133$.

Statistical analysis by means of F and t tests (P =95%) revealed no significant difference either for the slopes (parameter estimate = 0.01, limit value = 4.6) or the y-intercepts (parameter estimate <0.01, limit value = 4.6). The calibration curves were not significantly different from each other. This means that the excipient content of the tablet does not interfere with the active ingredient determination and the accuracy is thereby verified.

To determine precision, 100 μl of a test solution (equivalent to 100 ng of brotizolam)

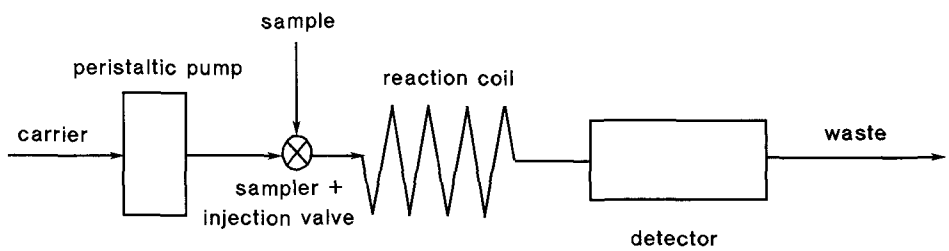

Figure 6
Flow diagram for brotizolam determination.

was injected 15 times into the FIA apparatus and the standard deviation was determined from the peak area. The RSD was 0.79%. The limit of quantitation was determined by the method of Currie. For brotizolam this value was 5 ng.

The sample processing rate achievable with the method described is 200 sample injections per h. This proves that FIA is a potential method for dissolution rate testing and is distinguished by its high flexibility, easy adaptability to dissolution testers and cost-effective instrumentation.

Conclusions

Manual testing of active ingredient release from oral dosage forms using the paddle method is the most time-consuming operation in the analytical characterization of drugs. Filling, buffer change (for controlled release drugs), emptying and cleaning operations in particular are mindless activities for laboratory staff. Automation of these operations results not only in more streamlined performance of dissolution testing but also in standardization of the techniques. Complete automation releases laboratory staff for more valuable tasks.

Combining the various analytical measurement techniques mentioned with the AUTO DISS system increases the flexibility of the system. Specific but 'slow' chromatographic techniques are supplemented by photodiode-array spectrophotometry and FIA which are extremely fast and economical methods.

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