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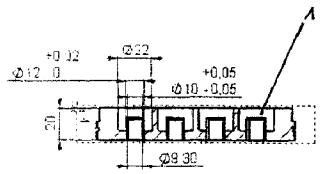
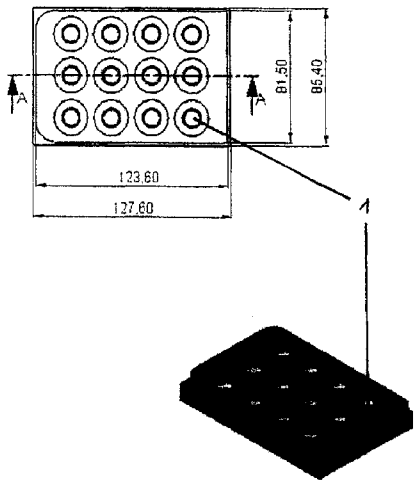
(54) Title: METHOD AND COMPLETE SYSTEM FOR DEVELOPING FORMULATIONS AND THE IN VITRO TESTING THEREOF WITH GOOD PREDICTABILITY OF THE ABSORPTION IN VIVO, HIGH THROUGHPUT AND LOW REQUIREMENT OF ACTIVE SUBSTANCE

Special microtitre plate according to the invention for direct UV measurement





(57) Abstract: The invention relates to a method of developing galenic formulations, preferably solid oral preparations, characterised by the parallel combinatory preparation of a plurality of liquid formulations or solutions on a tiny scale, methods of determining the supersaturation behaviour of the solutions and release characteristics of the solid formulations, as well as a special microtitre well plate which can be used in the above-mentioned method and which is suitable for carrying out UV measurements.

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Method and complete system for developing formulations and the in vitro testing thereof with good predictability of the absorption in vivo, high throughput and low requirement of active substance

Introduction

The present invention relates to a rational method of developing galenic formulations, preferably oral galenic formulations, based on a dissolution method with a high throughput and low consumption of active substances, as well as the apparatus required therefor, consisting of a specific combination of pieces of equipment, some of which are already known.

Background to the invention

The prerequisite for the absorption of active substances is substantially total dissolution in the gastrointestinal tract. In the case of poorly soluble active substances, only a small proportion of the active substance dissolves when conventional formulations are administered orally and frequently there are absorption problems.

The poor solubility can be determined as follows:
The influence of the dose of the particular active substance on its bioavailability can be described quantitatively by the concept of the (dimensionless) dose number "Do". This is defined as:

$$\mathbf{Do = (Mo / Vo) / Cs},$$

where

Mo = dose (mg),
Vo = volume of liquid present (ml) and
Cs = saturation solubility (mg / ml).

According to an assumption which is now generally accepted, the volume of liquid in the stomach after taking a preparation is approx. 250 ml. (Löbenberg, R., Amidon, G.L.: Modern bioavailability, bioequivalence and biopharmaceutics classification system.

New scientific approaches to international regulatory standards (Eur. J. Pharm. Biopharm. 50 (2000) 3-12).

With dosages that give a dose number of less than 1, no solubility problems arise. Only if the critical dose number of 1 is exceeded may there be relevant deteriorations in solubility and hence a reduction in bioavailability. -As a rule, however, the real problem area only starts at dosages that give a dose number significantly higher than 1, as at least some of the dissolved substance is constantly removed from the equilibrium by the absorption process.

Table 1 shows by way of example an active substance which is readily soluble in an acidic medium but poorly soluble at a higher pH. Up to pH 3 total dissolution occurs at the dosage of 150 mg / person taken, whereas at pH 6 less than 1 % of the dose dissolves, i.e. in an anacid stomach considerable absorption problems can be expected.

Table 1: Dependency of the dose number on the solubility

pH	**solubility** [mg/ml]	**Do** (volume 200 ml)
1	80	0.01
2	5.8	0.13
3	0.8	0.94
4	0.08	9.38
5	0.034	22.1
6	0.0045	166.7
7	0.0036	208.3
8	0.0021	357.1

Substantially total absorption may be achieved by the addition of suitable excipient combinations which eliminate the solubility problems. This applies to both instant and delayed-release formulations, while particularly when developing delayed-release formulations on the one hand the formulation must ensure that the active substance also dissolves completely in the lower sections of the bowel, but on the other hand an optimum delayed-release is also achieved in each case.

Prior art

Formulations are currently produced in conventional apparatus for various technologies and then tested in vitro using dissolution methods. Admittedly, in recent years, a number of manufacturers have put equipment on the market designed for smaller batch sizes, but with the apparatus currently on sale the smallest amounts of formulations that can be produced therewith are generally 10 – 50 g, as shown in Table 2.

Table 2: Minimum manufacturing sizes using different manufacturing technologies

production step	minimum batch size
direct tableting	approx. 10 gm
wet granulation	50 gm
melt granulation	50 gm
melt embedding	approx. 10 gm
fluidised bed granulation	10 gm
pellet manufacture	50 gm
tableting	1 tablet
extrusion	20 gm

The dissolution characteristics of these formulations are currently tested in Dissolution Tests using standardised methods (USP methods), which have the following characteristics:

- use of non-physiological high volumes, usually 500 or 900 ml;
- The test is carried out under so-called "sink conditions" , i.e. the active substance has to dissolve completely in the test medium;
- Standard commercial release apparatus made by various manufacturers is used (e.g. Sotax Varian etc.), which is complicated to clean in each case, on account of the complex structure (temperature-controlled bath, 6-8 release pots, complex hose system for pumping round into the measuring cells of the photometer etc.) , i.e. the throughput is relatively low.

These test methods are admittedly highly suitable for the quality control of formulations, but do not allow any reliable pronouncements to be made regarding in vivo absorption in the case of formulations of poorly soluble active substances, as the sink conditions are not achieved. Furthermore, the currently conventional manufacturing and testing methods have major disadvantages for poorly soluble active substances, as they require large amounts of active substance, allow only a low throughput and the test methods have little predictive power for the absorption in the patient.

Problem of the invention

It is sufficient, for substantially total absorption, if largely total release of the active substance is achieved in vitro within 15 to 30 minutes at all the pH values that occur physiologically and the active substance remains dissolved for approx. 10 - 30 minutes. If these conditions are met, substantially total absorption is virtually guaranteed with theoretically good absorbability of the active substance, i.e. the absolute bioavailability is reduced only by first pass effects, but not by incomplete absorption. If this goal is not achieved entirely, for example because insufficient supersaturation has been obtained or because other aspects such as stability, dose or safety had to be taken into account, good absorption may still be obtained, but the risk of "absorption failure" occurring is increased. Particularly careful in vivo testing (e.g. making the test subjects anacid, testing on older patients), while taking account of the later patient situation, is essential in these cases.

With delayed-release forms, the release of the active substance must be achieved at all the physiologically occurring pH values in the intestinal tract and the active substance must remain in solution long enough to ensure absorption even in the case of longer diffusion paths at lower sections of the bowel.

The aim of the present invention is to provide a rational method for developing galenic formulations, preferably oral galenic formulations, based on a dissolution method which has the advantages of a high throughput, the use of small amounts of active substance or

formulation and good predictability for the absorption in the patient, as well as the apparatus required for this purpose.

A low consumption of active substance is of considerable advantage, as at the early stages of the development of new active substances ("new chemical entities", NCEs) because of the often very time-consuming processes for synthesising increasingly more complex molecules generally only small amounts of active substance are available.

Brief summary of the invention

The invention relates to a **Method A** for developing galenic formulations, preferably solid oral preparations, characterised by the parallel combinatory preparation of a number of liquid formulations or solutions on a tiny scale, and testing the supersaturation characteristics.

A preferred **embodiment B** of the invention comprises in one step testing the supersaturation behaviour of solutions instead of the otherwise conventional dissolution tests on solid forms, particularly testing the stability of a supersaturated active substance solution by adding buffer at the pH of minimal solubility of the active substance.

A second preferred **embodiment C** of the invention comprises in one step the production of a thin solid film of a solid formulation by eliminating the liquid constituents from a liquid formulation and investigating the dissolution characteristics of the solid film, particularly testing the dissolution characteristics of the solid film produced directly by evaporation in the release vessels, instead of the otherwise conventional dissolution tests on solid forms.

A third preferred **embodiment D** of the invention comprises in one step testing the release characteristics in an in vitro release model using physiological volumes, the testing preferably being carried out at the pH of the minimum solubility of the active substance, if this is in the physiological range.

A further **object E** of the invention is a special microtitre plate, consisting of a plate with a plurality of regularly arranged, preferably cylindrical recesses, formed in this plate from above, which differs from conventional microtitre plates in that instead of the complete bore each recess (well) comprises an elevation formed in the recess from the underside of the plate, preferably in the form of an inner cylinder which is lower than the bore and terminates at the top in a UV-permeable quartz disc (cf. **Figure 7**, Appendix). These special microtitre plates may for example be milled from a piece of plastics, the quartz disc is pressed tightly into a ring of precisely the correct size. Alternatively these special microtitre plates may for example also be produced by injection moulding from UV-permeable plastics.

Thus, by suitable fill levels, it is possible to obtain layer thicknesses above the quartz plate which are suitable for direct UV measurements (e.g. 0.8 – 2 mm leading to extinction values in the range from 0.5 to 2). Highly accurate layer thicknesses can be obtained by means of a precisely fitting mating piece with an inverse structure (lock and key principle), which fits directly into the special microtitre plate and when pushed fully together results in the desired layer thickness.

Detailed description of the invention

Within the scope of **Method A** according to the invention,- mixtures of the active substance with various excipients may be prepared for example by an automated process in a plurality of parallel experiments on a tiny scale by combining the individual components sampled in parallel fashion from a plurality of storage vessels in a plurality of test vessels, e.g. by pipetting the individual components together from microtitre storage plates into microtitre test plates.

The terms "microtitre plate" and "well plate" are to be taken as synonymous for the purposes of the present invention, the term "well" referring to the depressions in the microtitre plate.

The expression "on a tiny scale" refers to the use of small amounts of active substance as well as small volumes of the liquid components, the ratio of active substance to

release volume always being chosen so that the highest human dose would be released in a volume of 200 ml. The reduction in the amount of active substance is determined by the small release volumes, i.e. the saving of active substance can be estimated directly using the volume ratios shown in Table 3:

If a dosage range of from 1 to 500 mg is taken as the human dose, then for each measurement 1 to 500 mg active substance are needed using standard methods, whereas with the methods according to the invention an amount of 2.5 ml of release medium needs only 0.0027 mg or 1.333 mg, 0.22 ml release medium requires only 0.0002 mg or 0.12 mg, i.e. less than 1/1000 of the original dose.

By preparing the formulations as solutions and testing the supersaturation behaviour by adding buffer at the pH of minimum solubility of the active substance a particularly high sample throughput can be achieved.

For determining the optimum composition, the recipes are produced directly in the release vessel, e.g. in a 96 well plate on a tiny scale (1 - 10 mg active substance/mixture) by pipetting together pre-prepared, usually aqueous solutions (excipient and active substance) or suspensions. If necessary the active substance may also be dissolved in small amounts of organic, water-miscible solvent.

Suitable devices for this purpose are pipetting robots. Solutions of active substance and excipient are placed in 96 wells (normal height with 250 µl/well microtitre plates or deep well plates containing 2.5 ml/well in each case).

The saving on substance achieved by this method is shown in Table 3. It will be apparent that the substance saving is up to a factor of 1000.

Table 3: Active substance requirements in various dissolution tests

	volume [ml]	amount of active substance [mg]
Standard	**900**	**100**
Standard "BCS"	**900**	**450**
Standard	**500**	**100**
Standard "BCS"	**500**	**250**
24 well plate	**2.4**	**1.2**
96 well plate	**0.2**	**0.1**

BCS = Biopharmaceutics classification system.

The throughput is also higher by powers of ten: Whereas in the preparation of solid recipes generally approx 1 to 5 per day are possible, here 96 recipes can be produced in a few minutes.

An example of the subdivision of a microtitre storage plate, with which a simple pipetting programme can be carried out, is shown in **Figure 1** (Appendix).

The abbreviations used specifically indicate:

Wa: water; Wi: active substance solution;

S1 – S4 physiologically acceptable acids, which may be used in formulations; H1 – H24 physiologically acceptable excipients, which may be used in formulations for improving supersaturation characteristics.

An example of the subdivision of a 96-well microtitre test plate is shown in **Figure 2** (Appendix). All the wells also contain active substance solution.

The next test plates would contain the remaining mixtures of the acids 2 – 4 with the corresponding excipients. By changing the solutions pipetted in, it is thus possible to add different amounts of excipients, and similarly complex combinations of all kinds of excipients can also be prepared in this way.

Theoretically, instead of 96-well plates it is also possible to use other formats such as 12- or 24-well plates, but the drawback is the larger amount of active substance needed.

The **embodiment B** of the invention comprises in one step testing the supersaturation behaviour of the solutions prepared by **Method A**-, instead of the otherwise conventional dissolution tests on solid forms, particularly testing the stability of supersaturated active substance solutions by adding buffer at the pH of minimum solubility of the active substance. One example of testing the supersaturation behaviour with various excipients is shown in the precipitation experiments illustrated in **Figure 3** (Appendix) .

The excipient is found to have a significant influence in terms of supersaturation, the most suitable excipients keeping the active substance fully in solution.

Embodiment C relates to the production of a thin solid film of a solid formulation (preparation of "solid recipes in the test tube") by eliminating the liquid constituents of a liquid formulation prepared by **Method A,** for example, and investigating the dissolution characteristics of the solid film, particularly testing the dissolution characteristics of the solid film produced directly by evaporation in the release vessels, instead of the otherwise conventional dissolution tests on solid forms.

The recipe is first of all produced as described hereinbefore with reference to **Method A**, preferably using specially made 24 or 12 well plates instead of 96 well plates. The recipes thus obtained (total volume < 1 ml) are then evaporated down, for example, under reduced pressure, possibly in the Rotavapor, wherein the aqueous solutions contained in the special microtitre plate are evaporated down with agitation and heating, while a film only a few microns thick is formed on the walls of the wells. This can then be used immediately for the release so as to determine the dissolution characteristics. The only important factor here is the speed of dissolution of the recipe, but not the breakdown of a larger solid preparation, which in conventional solid forms such as tablets would be superimposed on the dissolution process and in some circumstances would even dominate it.

This procedure requires only small amounts of active substance and excipients, can be carried out exceptionally quickly and also has the following important advantage: a~~A~~s the recipe is "applied" to the test tube wall in a very thin layer, the "breakdown time" of the recipe plays only a minor part, i.e. the intrinsic properties of the excipients with regard to dissolving the active substance and keeping it in solution can be determined directly.

Embodiment D of the invention relates to testing the release characteristics of solid galenic formulations, for example the solid films of embodiment C, in an in vitro release model using physiological volumes, while the relationship between the highest dose of active substance and the volume of release medium is guided by the amount drunk in the human trial or in the patient. For example, with an assumed dose of approx. 120 mg dose and a drink volume of approx. 240 ml, in the test approx. 0.5 mg of active substance are used to approx. 1 ml of release volume. This is preferably done at the "least favourable pH "-, i.e. the pH of minimum solubility of the active substance, if this is in the physiological range, instead of using pH values which correspond to sink conditions, i.e. total solubility of the active substance. For the testing, conventional physiologically acceptable buffers of low buffer capacity in the range from 0.05 – 0.005 are preferably used, which roughly correspond to the physiological conditions, e.g. phosphate, citrate or carbonate buffers.

The testing is preferably carried out by direct UV measurement in the release medium in 12, 24 or 96 well plates instead of large-capacity release vessels, without centrifugation or filtration, so as to avoid false-negative release values. In addition, the UV measurement in the release medium is preferably carried out at a number of characteristic wavelengths which allow the true UV signal and the turbidity fraction to be evaluated by computation. The computation is preferably carried out by computerised determination of the extinction and turbidity fraction using the Excel Solver. It is advantageous to compress the release data onto characteristic values which allow rapid evaluation of the supersaturation behaviour of a plurality of formulations and thus permit a quick decision as to further formulation steps.

Preferred test strategies within **embodiment D** may be summarised under the following headings:

- Use of "physiological volumes", i.e. the relationship between the maximum dose of active substance and the volume of release medium should be similar to the amount of drink in the human trial or in the patient (e.g. at a dose of 120 mg and a drink volume of 240 ml, 0.5 mg of active substance should be used per 1 ml of release volume);
- "Least favourable pH", i.e. testing at the pH of the minimum solubility, if it is within the physiological range;
- Moderate buffer strengths in the range from 0.1 to 0.001, preferably 0.05 to 0.005;
- Moderate stirring or shaking;
- Measuring the "release" by UV measurement with evaluation of the total spectrum;
- With an active substance having very poor solubility some of the active substance is precipitated, or does not dissolve completely, i.e. the UV spectrum is a superimposing of dissolved active substance and turbidity. Conventional separation processes such as centrifugation or filtering often destroy the unstable supersaturation and would thus lead to false-negative predictions as to the absorbability of formulations. Therefore, the absorbable active substance fraction is calculated as described hereinafter under the heading "Evaluation of the UV spectra" ;
- By comparing the precipitation and dissolution characteristics it is possible to estimate whether a certain delayed-release effect on solid medicaments can already be expected as a result of the excipients chosen.
- Use of single- or multi-channel Elisa readers instead of the otherwise conventional photometers in order to allow in vitro releases with exceptionally small volumes (e.g. 200 µl in the 96-well plate) ;
- Use of temperature-controllable agitators instead of the large-capacity stirrers, in order to allow in vitro releases with exceptionally small volumes (e.g. 200 µl in the 96-well plate) ;
- Use of a semi-automatic apparatus in which the transfer of the well plates from the pipetter to the agitator or Elisa reader is carried out manually;

- Use of a fully automatic apparatus in which also all the transfers of the well plates are carried out by a robot;

Whereas it is generally assumed that the precipitation from a supersaturated solution is a hardly reproducible, highly fluctuating process, extensive precipitation tests have shown that under the standardised conditions according to the invention the in vitro data can be reproduced very well during precipitation. Thus, this method is highly suitable for the early optimising phases.

Practical procedure, equipment needed:

- The releases or precipitation characteristics are tested directly in the plates in which the recipe was produced;
- pipetting in of buffer maintained at a temperature of 37°C (release medium: 0.2 ml for 96-well plates or 2.4 ml for 24-well plates) using an automatic pipetting machine with a time delay matched to the measuring speed;
- temperature adjustment and agitation in temperature-controlled agitators;
- fixed time scheme for the measurements, e.g. 5, 10, 15, 20, 25 and 30 min, for rapidly released formulations;
- for delayed-release forms, suitably modified schemes are used, e.g. 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48 hours;
- removal of 15 – 20 µl aliquots from the test plate in 384 (or 40-70 µl in 96) well measuring plates;
- UV quantification of the measuring plates with multichannel Elisa readers at several wavelengths in order to be able to distinguish between "dissolved" active substance and turbidity; exporting of the data into table calculation programmes;
- evaluation using table calculation programmes, automatic generation of the results tables, e.g. using the pivot-tables function, in which release curves can also be generated directly, and optionally plots, e.g. using table calculation programmes such as e.g. Excel.

Evaluation of the UV spectra

Figure 4 (Appendix) shows an example of an active substance, wherein the supersaturation of the active substance is destroyed by centrifuging.

A method was therefore sought by which the "true absorption of active substance " and hence the releases could also be calculated. This may be attempted to some extent using commercial evaluation software for photometers or dissolution apparatus, if they include options such as forming the first and second derivative or a "scatter correction". However, the problem then arises that the residual error is very difficult to estimate. Finally, the following evaluation approach proved more suitable:

- The absorption is measured at a number of wavelengths, e.g. with a UV spectrum as shown in **Figure 5** (Appendix), at the maximum (300nm) and minimum (270nm) of the UV spectrum of the active substance and at values at which the dissolved active substance exhibits little (330nm) or no (360 + 400 nm) absorption;
- for the UV characteristics of the turbidity a mathematical function, e.g. linear (equation y = a*x + b), quadratic (equation y = a*x^2+ b*x +c), exponential (equation y = a+e^(-b*lambda)) is assumed
- for the UV data the relative absorption fractions of the particular wavelengths are used, e.g. for the case described in Figure 5, for the wavelength 275 nm the ratio of the extinction at 275 nm to the extinction at 300 nm would be used
- for each wavelength an equation system is established, consisting of the sum of the UV absorption and the turbidity;
- The sum of the distance squares between the absorption calculated and measured is formed;
- this sum is minimised with the aid of the Excel Solver, by using, as the cells which are to be optimised, the factor for the extinction and the parameters of the turbidity (e.g. in the linear case these are the axial distance and the gradient of the regression line);
- As in the case described there are 5 equation systems for only 3 unknowns, the desired parameters can easily be determined;
- the "true release" is calculated from the factor of the extinction.

A precise description of the calculation method is given in Example 5.

The reliability of this procedure was determined as follows:

- A molecularly disperse solution of an active substance was prepared;
- As turbidity a suspension of small-particled latex particles was prepared;
- Then various mixtures of active substance solution and latex suspension were prepared and their absorption at the characteristic wavelengths was measured;
- Evaluation was carried out using the Excel Solver;
- The values measured were compared with the theoretical values known from the mixing ratio.

The UV spectra of some mixtures by way of example and the comparison of the measured spectrum as against the theoretical values which were calculated by the addition of the individual spectra are shown in **Figure 5** (Appendix).

The correlation of the data sets with the "true" extinction values calculated with Excel Solver with the theoretical values known from the mixing ratio are shown in **Figure 6** (Appendix).

As the gradient and the correlation coefficient are close to the ideal value of 1 and the axis intercept is negligible, the reliability of this method of evaluation is proven.

Choice for further optimising of the recipe:

In order to enable decisions to be taken quickly for further refining the formulations, in view of the large number of test series and the release curves, which are highly complex in parts, compressed data such as e.g. the area under the release curve for the desired investigation period and/or the maximum release are automatically included in the calculations for the evaluation. Once these values have been sorted in ascending order in a Table, the best formulations are at the lower end of the Table and a decision on further refinement can be taken immediately the release has ended. If a more accurate comparison of the release characteristics for different forms is required, it is advisable to compile the mean values of the release figures.

Although the in vitro release method according to the invention in no way simulates the situation in vivo, it has hitherto proved its worth as a good tool for optimising galenic recipes, as in all the cases tested good release values in vitro also resulted in very good absorption in vivo .

In vitro releases of multiparticulate solid forms

Using the well plate method described it is also possible to measure the releases of multiparticulate systems such as pellets, extrudates, microtablets, etc. In addition to the advantages already described, such as high throughput, low substance requirement, etc., it is also possible to use this method to determine the homogeneity of the individual particles. This is an important tool for refining the manufacturing processes, e.g. for determining the optimum spray parameters for active substance pellets, etc.

New well plates for direct measurement of the release or for precipitation tests

Instead of the transfer from the test well plate into the measuring well plate it is also possible to use a specially developed 12 or 24 well plate which allows direct measurement with small layer thicknesses. This is usually only possible with expensive fibre optics, which have come onto the market in recent years for the dissolution method.

Object E of the invention is a special well plate (special microtitre plate), consisting of a plate with a plurality of regularly arranged preferably cylindrical depressions formed in this plate from above, which differs from conventional microtitre plates in that instead of the full bore each depression (well) has an elevation formed in the depression from the underside of the plate, preferably in the form of an inner cylinder which is lower than the bore and terminates at the top with a UV permeable quartz disc (cf. **Figure 7**, Appendix). These special microtitre plates may for example be milled from a piece of plastics, the quartz disc is pressed tightly into a ring of precisely the correct size. Alternatively these special microtitre plates may for example also be produced by injection moulding from UV-permeable plastics.

Thus, by suitable fill levels, it is possible to obtain layer thicknesses above the quartz plate which are suitable for direct UV measurements (e.g. 0.8 – 2 mm leading to extinction values in the range from 0.5 to 2). Highly accurate layer thicknesses can be obtained by means of a precisely fitting mating piece with an inverse structure (lock and key principle), which fits directly into the special microtitre plate and when pushed fully together results in the desired layer thickness.

Object E of the invention may for example be a 12 or 24 well plate, as shown in **Figure 7** (Appendix), and have the structure described as follows:

The external dimensions and the dimensions of the wells correspond to commercially obtainable well plates, which means that all the otherwise conventional equipment such as agitators, Elisa readers etc can be used without the need for modification. UV measurement with small layer thicknesses is made possible by an inner cylinder which terminates at the top in a UV permeable quartz disc. The desired layer thickness can be adjusted by means of the amount of buffer packed in. Even greater precision is achieved by a low-rise mating part (cover) of analogous construction which fits according to the lock and key principle.
The plate may be used both for precipitation tests and for dissolving solid forms. A particular advantage of solid forms is that the still solid fractions are in the bottom annular part and thus do not interfere with the measurement.

In practical use, the plate is agitated in a temperature-controlled agitator and then measured in an Elisa reader at the respective measuring times.

Brief legend to the Figures:

Figure 1 shows an example of the subdivision of a microtitre storage plate which enables a simple pipetting programme to be carried out.

Figure 2 shows an example of the subdivision of a 96-well microtitre test plate. All the wells also contain active substance solution.

Figure 3 shows an example of testing the supersaturation behaviour with various excipients by precipitation tests according to **embodiment B** of the invention. The differing supersaturation characteristics caused by various excipients can be detected.

Figure 4 shows UV spectra of an active substance in the dissolution test, in which the supersaturation of the active substance is destroyed by centrifuging.

Figure 5 shows measured and theoretical UV spectra of a dissolved active substance and the turbidity suspensions thereof.

Figure 6 shows the correlation of the data sets of "true" extinction values calculated with Excel Solver with the theoretical values known from the mixing ratio for dissolved active substance and the turbidity suspensions thereof.

Figure 7 shows, as an example of a special well plate according to the invention (special microtitre plate milled from one piece; Object E of the invention), a 12 well plate for direct UV measurement, shown once in longitudinal sectional view along an axis A-A, and once in plan view, and in photographic view from diagonally above. All the measurements are given in mm and are intended solely by way of example.
Reference numeral: 1: UV permeable quartz disc

The following Examples serve to illustrate the invention without restricting it:

Example 1: Testing the supersaturation with various excipients for an active substance which is readily soluble in the acidic range but virtually insoluble at pH values above 5; assumed human dose 100 mg, assumed volume in humans is 200 ml

The operational steps for a semiautomatic apparatus are as follows:

- The active substance solution used is a solution of 11.5 mg/ml active substance in 1n hydrochloric acid, the excipients are dissolved in water in the concentrations 11.5 mg/ml, 3.45 mg/ml, 11.5 mg/ml and 0.345 mg/ml;

- active substance and excipient solutions are transferred into a 96 deep-well plate analogously to Figure 1 as the storage well and placed in an automatic pipetting apparatus at the "storage plate" position;
- a 96 well plate with a capacity of 300 µl per well is placed in an automatic pipetting apparatus at the "test plate" position;
- 10 µl aliquots of active substance solution and various excipient solutions are transferred from the storage plate into the first 4 rows of a test plate with the automatic pipetting apparatus according to a pipetting programme (in each case 8 wells are filled simultaneously using a pipetting head of the 8 needles or 8 tips);
- The test plate is then brought to 37°C in a temperature-controlled agitator and mixed thoroughly;
- 200 µl of a 0.01 M phosphate buffer maintained at a temperature of 37°C and adjusted to pH 5.5 are transferred with an 8-fold pipette into the first 4 rows of the test plate with agitation, the time interval between the rows corresponding to the measuring time of the UV measurement;
- after the measuring intervals laid down in each case the test plates are again placed in the automatic pipetting apparatus and 20 µl aliquots are transferred into a 384 well measuring plate and then in each case measured in a multichannel Elisa reader at 5 different wavelengths;
- after the end of a measuring series, e.g. after 5, 10, 15, 20, 25 and 30 minutes, all the UV data are exported into a table calculation programme, e.g. Excel;
- then the data of the "true UV extinction" are calculated using the Excel Solver for all the wells and times and from these the contents which have remained in solution are calculated using a standard;
- then data compression is carried out, e.g. calculation of the area under the time/solution curve (AUC values) using known methods;
- after a number of measuring series have been carried out in which various concentrations of all the excipients to be used have been tested, all the AUC data are transferred into a file and sorted. These results are used to determine how to optimise the process further.

Example 2: Testing the supersaturation with various excipients for an active substance which is readily soluble in the acidic range but virtually insoluble at pH values above 5; assumed human dose 100 mg, assumed volume in humans is 200 ml

- The active substance solution used is a solution of 11.5 mg/ml active substance in 1n hydrochloric acid, the excipients are dissolved in water at concentrations of 11.5 mg/ml, 4.45 mg/ml, 1.15 mg/ml and 0.5 mg/ml;
- 10 µl of active substance solution and different excipient solutions are filled deeply into a special microtitre plate, then the water is evaporated using the Rotavapor described previously and then the temperature is readjusted to 37 °C.
- 2.4 ml of a 0.01 M phosphate buffer maintained at a temperature of 37°C and adjusted to pH 5.5 are transferred with a Multipette into wells of the special microtitre plate with agitation, the time interval between the rows corresponding to the measuring time of the UV measurement;
- after the measuring intervals laid down in each case the test plates are again placed in the automatic pipetting apparatus and 20 µl aliquots are transferred into a 384 well microtitre measuring plate (or 50 µl aliquots are transferred into a 96-well microtitre measuring plate) and then in each case measured in a multichannel Elisa reader at 5 different wavelengths;
- after the end of a measuring series, e.g. after 5, 10, 15, 20, 25 and 30 minutes, all the UV data are exported into a table calculation programme, e.g. Excel;
- then the data of the "true UV extinction" are calculated using the Excel Solver for all the wells and times and from these the contents which have remained in solution are calculated using a standard;
- then data compression is carried out, e.g. calculation of the area under the time/solution curve (AUC values) using known methods;
- after a number of measuring series have been carried out in which various concentrations of all the excipients to be used have been tested, all the AUC data are transferred into a file and sorted. These results are used to determine how to optimise the process further.

Example 3: Testing the dissolution characteristics of small-particled solid forms for an active substance which is readily soluble in an acidic and/or basic medium, but poorly soluble at pH values from 4 - 8 (assuming a human dose of 100 mg active substance)

The operating steps for a semiautomatic apparatus are as follows:

- From 6 different formulations, in each case 2x as many mg of a small-particled solid form are weighed out such that the total amount corresponds to a dose of 12 mg, i.e. for an active substance content of 25 % this would be 48 mg of the formulation;
- The formulations weighed out are placed in the first three rows of a 24 well plate and heated to 37 °C in a temperature-controlled agitator;
- 2.4 ml of a 0.01 M phosphate buffer maintained at a temperature of 37°C and adjusted to pH 5.5 are transferred with a Multipette into the first 3 rows of the test plate with agitation, the time interval between the rows corresponding to the measuring time of the UV measurement;
- after the measuring intervals laid down in each case the 24-well plate is placed in the automatic pipetting apparatus and 20 µl aliquots are transferred into a 384 well measuring plate (or 50 µl aliquots are transferred into a 96-well measuring plate) and then in each case measured in a multichannel Elisa reader at 5 different wavelengths;
- after the end of a measuring series, e.g. after 5, 10, 15, 20, 25 and 30 minutes, all the UV data are exported into a table calculation programme, e.g. Excel;
- then the data of the "true UV extinction" are calculated using the Excel Solver for all the wells and times and from these the contents which have remained in solution are calculated using a standard;
- then data compression is carried out, e.g. calculation of the area under the time/solution curve (AUC values);
- after a number of measuring series have been carried out in which various concentrations of all the excipients to be used have been tested, all the AUC data are transferred into a file and sorted. These results are used to determine how to optimise the process further.

Example 4: Testing the dissolution characteristics of small-particled solid forms for an active substance which is readily soluble in an acidic or basic medium, but poorly soluble at pH values from 4 - 8 (assuming a human dose of 100 mg active substance) using the special well plate according to the invention

The operating steps for a semiautomatic apparatus are as follows:

- From 6 different formulations, in each case 2x as many mg of a small-particled solid form are weighed out such that the total amount corresponds to a dose of 14 mg, i.e. for an active substance content of 25 % this would be 56 mg of the formulation;
- The formulations weighed out are placed in the annular depressions of the special well plate and heated to 37 °C in a temperature-controlled agitator;
- 2.4 ml of a 0.01 M phosphate buffer maintained at a temperature of 37°C and adjusted to pH 5.5 are transferred with a Multipette into the 12 depressions of the special well plate with agitation, the time interval between the rows corresponding to the measuring time of the UV measurement;
- after the measuring intervals laid down in each case the special well plate is measured directly in a single-channel Elisa reader at 5 different wavelengths;
- after the end of a measuring series, e.g. after 5, 10, 15, 20, 25 and 30 minutes, all the UV data are exported into a table calculation programme, e.g. Excel;
- then the data of the "true UV extinction" are calculated using the Excel Solver for all the wells and times and from these the contents which have remained in solution are calculated using a standard;
- then data compression is carried out, e.g. calculation of the area under the time/solution curve (AUC values);
- after a number of measuring series have been carried out in which various concentrations of all the excipients to be used have been tested, all the AUC data are transferred into a file and sorted. These results are used to determine how to optimise the process further.

Example 5: Description of the evaluation of the "true extinction" with the aid of the Excel solver using the example of a turbidity with linear dependency on the wavelength:

- The absorption is measured at a number of wavelengths, e.g. with a UV spectrum as shown in Figure 5, at the maximum (300nm) and minimum (270nm) of the UV spectrum of the active substance and at values at which the dissolved active substance exhibits little (330nm) or no (360 + 400 nm) absorption
- for the UV characteristics of the turbidity a mathematical function, e.g. linear (equation y = a*x + b, where x is the respective wavelength), is assumed
- for the UV data the relative absorption fractions of the particular wavelengths are used, e.g. for the case described in Figure 5, for the wavelength 275 nm the ratio of the extinction at 275 nm to the extinction at 300 nm would be used; in this case the factor is 0.732, for 330 nm the factor is 0.132, at 360 and 400 nm when the active substance is dissolved there is no absorption. In addition the extinction of the standard, which is at 1.97 in this example, is taken into account.
- For each wavelength an equation ~~sytems~~system is established, consisting of the sum of the UV absorption and the turbidity; this reads as follows:

  At 270 nm: y= a*270 +b +c*0.73*1.97

  At 300 nm: y= a*300 +b +c*1*1.97

  At 330 nm: y= a*330 +b +c*0.132*1.97

  At 360 nm: y= a*360 +b

  At 400 nm: y= a*400 +b
- The sum of the distance squares between the absorption values calculated as above and the absorption values measured is formed;
- this sum is minimised with the aid of the Excel Solver, by using, as the cells which are to be optimised, the factor c for the extinction and the parameters of the axial distance b and gradient a of the regression line of the turbidity;
- As in the case described there are 5 equation systems for only 3 unknowns (a, b and c), the desired parameters can easily be determined;
- the "true release" for the respective measuring time is calculated from the factor c of the extinction thus obtained, according to the formula % release is c*100/1.97.
- this calculation can be substantially automated using the Excel macro function.

For quadratic and exponential turbidities, the evaluation is carried out analogously by drawing up the corresponding equation systems.

By comparing the sum of the distance squares which remain as residual errors for the various turbidity characteristics after the Solver has been run, the turbidity characteristic which is most suitable for the particular problem can be determined: it is the method which has the lowest total sum of all the distance squares after a complete measuring series has been carried out.

Patent Claims

1. A **Method A** for developing galenic formulations, preferably solid oral preparations, characterised by the parallel combinatory preparation of a plurality of liquid formulations or solutions on a tiny scale, and testing the supersaturation behaviour.

2. Method for developing galenic formulations, preferably solid oral preparations, comprising in one step testing the supersaturation behaviour of the solutions instead of the otherwise conventional dissolution tests on solid forms, particularly testing the stability of a supersaturated active substance solution by adding buffer at the pH of minimum solubility of the active substance.

3. Method for developing galenic formulations, preferably solid oral preparations, comprising in one step producing a thin solid film of a solid formulation by eliminating the liquid constituents of a liquid formulation and investigating the dissolution characteristics of the solid film, particularly testing the dissolution characteristics of the solid film produced directly by evaporation in the release vessels, instead of the otherwise conventional dissolution tests on solid forms.

4. Method for developing galenic formulations, preferably solid oral preparations, comprising in one step testing the release characteristics of a solid formulation in an in vitro release model using physiological volumes, the testing preferably being carried out at the pH of the minimum solubility of the active substance, if this is in the physiological range.

5. Method according to claim 1, comprising in one step testing the supersaturation behaviour of the solutions instead of the otherwise conventional dissolution tests on solid forms, particularly testing the stability of a supersaturated active substance solution by adding buffer at the pH of minimum solubility of the active substance.

6. Method according to claim 1, comprising in one step producing a thin solid film of a solid formulation by eliminating the liquid constituents of a liquid formulation and investigating the dissolution characteristics of the solid film, particularly testing the dissolution characteristics of the solid film produced directly by evaporation in the release vessels , instead of the otherwise conventional dissolution tests on solid forms.

7. Method according to claim 1, comprising in one step testing the release characteristics of a solid formulation in an in vitro release model using physiological volumes, the testing preferably being carried out at the pH of the minimum solubility of the active substance, if this is in the physiological range.

8. Special microtitre plate, consisting of a plate with a plurality of regularly arranged, preferably cylindrical recesses, formed in this plate from above, characterised in that instead of the complete bore each recess (well) comprises an elevation formed in the recess from underneath the plate, preferably in the form of an inner cylinder which is lower than the bore and terminates at the top in a UV-permeable quartz disc.

Figure 1: Example of the filling of a storage plate, which allows a simple pipetting programme to be carried out.

well	1	2	3	4	5	6	7	8	9	10	11	12
A	Wa	Wi	S1	S1	S2	S3	S4	H1	H9	H17		
B	Wa	Wi	S1	S1	S2	S3	S4	H2	H10	H18		
C	Wa	Wi	S2	S1	S2	S3	S4	H3	H11	H19		
D	Wa	Wi	S2	S1	S2	S3	S4	H4	H12	H20		
E	Wa	Wi	S3	S1	S2	S3	S4	H5	H13	H21		
F	Wa	Wi	S3	S1	S2	S3	S4	H6	H14	H22		
G	Wa	Wi	S4	S1	S2	S3	S4	H7	H15	H23		
H	Wa	Wi	S4	S1	S2	S3	S4	H8	H16	H24		

Figure 2: Example of the filling of a test plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	2xWa	S1+Wa	S1+H1	S1+H1	S1+H9	S1+H9	S1+H17	S1+H17	S2+H1	S2+H1	S2+H9	S2+H9
B	2xWa	S1+Wa	S1+H2	S1+H2	S1+H10	S1+H10	S1+H18	S1+H18	S2+H2	S2+H2	S2+H10	S2+H10
C	2xWa	S1+Wa	S1+H3	S1+H3	S1+H11	S1+H11	S1+H19	S1+H19	S2+H3	S2+H3	S2+H11	S2+H11
D	2xWa	S1+Wa	S1+H4	S1+H4	S1+H12	S1+H12	S1+H20	S1+H20	S2+H4	S2+H4	S2+H12	S2+H12
E	2xWa	S3+Wa	S1+H5	S1+H5	S1+H13	S1+H13	S1+H21	S1+H21	S2+H5	S2+H5	S2+H13	S2+H13
F	2xWa	S3+Wa	S1+H6	S1+H6	S1+H14	S1+H14	S1+H22	S1+H22	S2+H6	S2+H6	S2+H14	S2+H14
G	2xWa	S4+Wa	S1+H7	S1+H7	S1+H15	S1+H15	S1+H23	S1+H23	S2+H7	S2+H7	S2+H15	S2+H15
H	2xWa	S4+Wa	S1+H8	S1+H8	S1+H16	S1+H16	S1+H24	S1+H24	S2+H8	S2+H8	S2+H16	S2+H16

Figure 3: Example of different supersaturation behaviours caused by various excipients

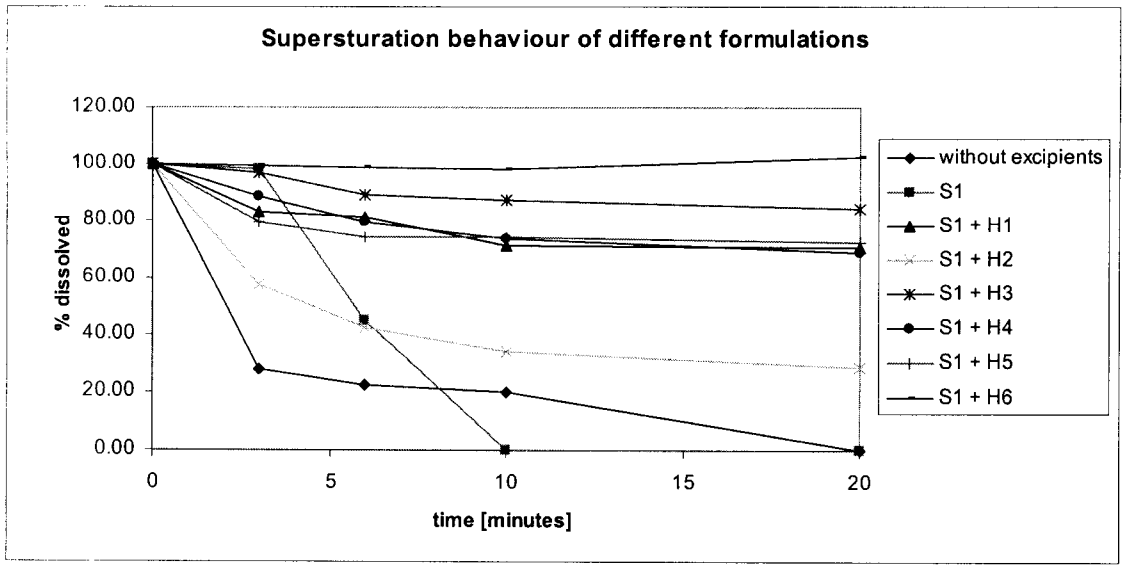


Figure 4: Comparison of the UV spectra of an active substance, wherein the supersaturation of the active substance is destroyed by centrifuging, without and with centrifugation

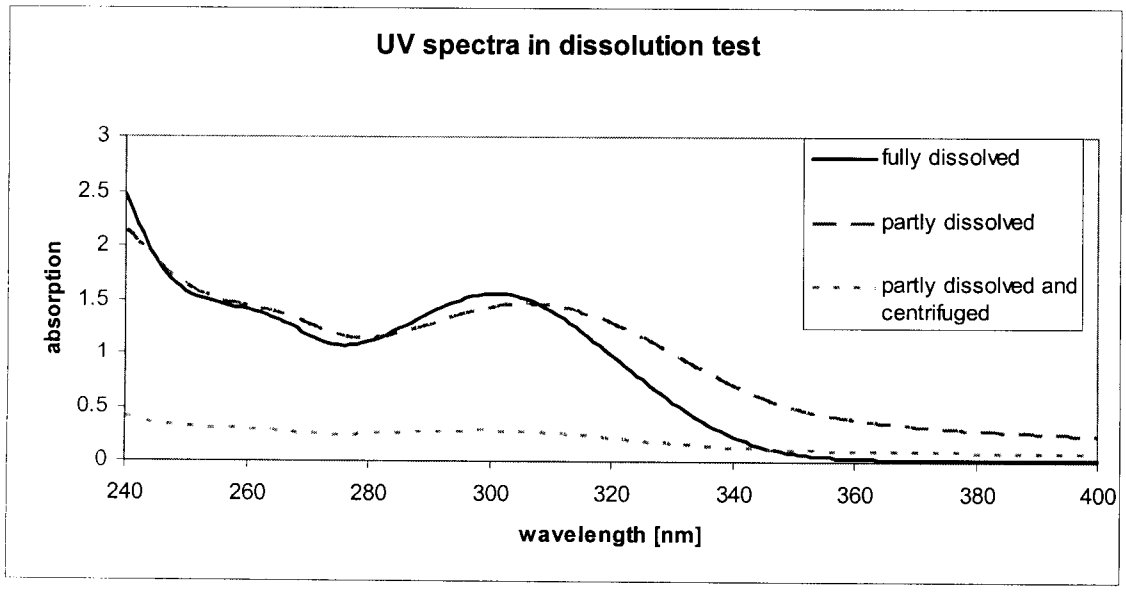


Figure 5: Comparison of the measured and theoretical UV spectra for dissolved active substance and a turbidity suspension

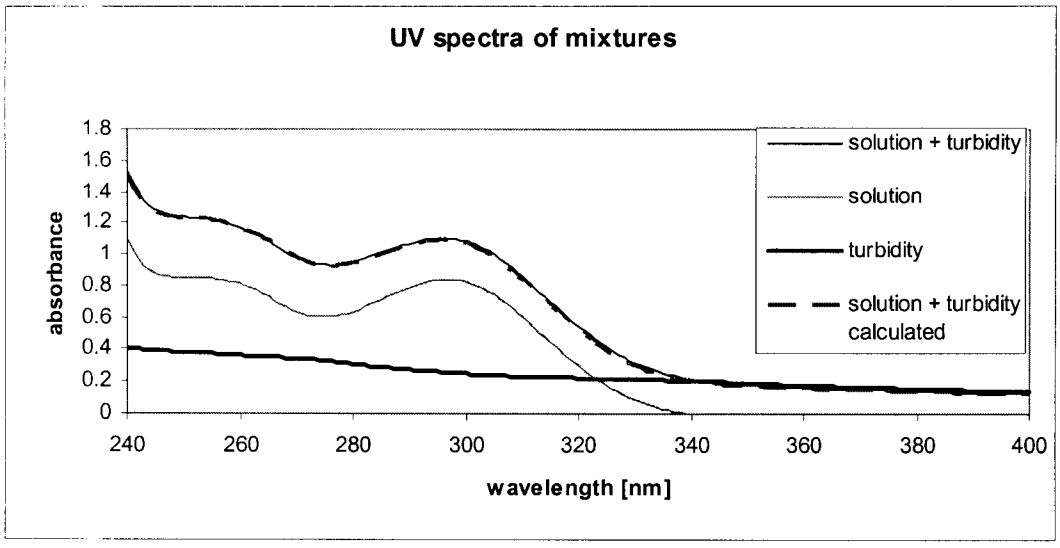


Figure 6: Comparison of the calculated and real extinction of various mixing ratios of dissolved active substance and turbidity suspension.

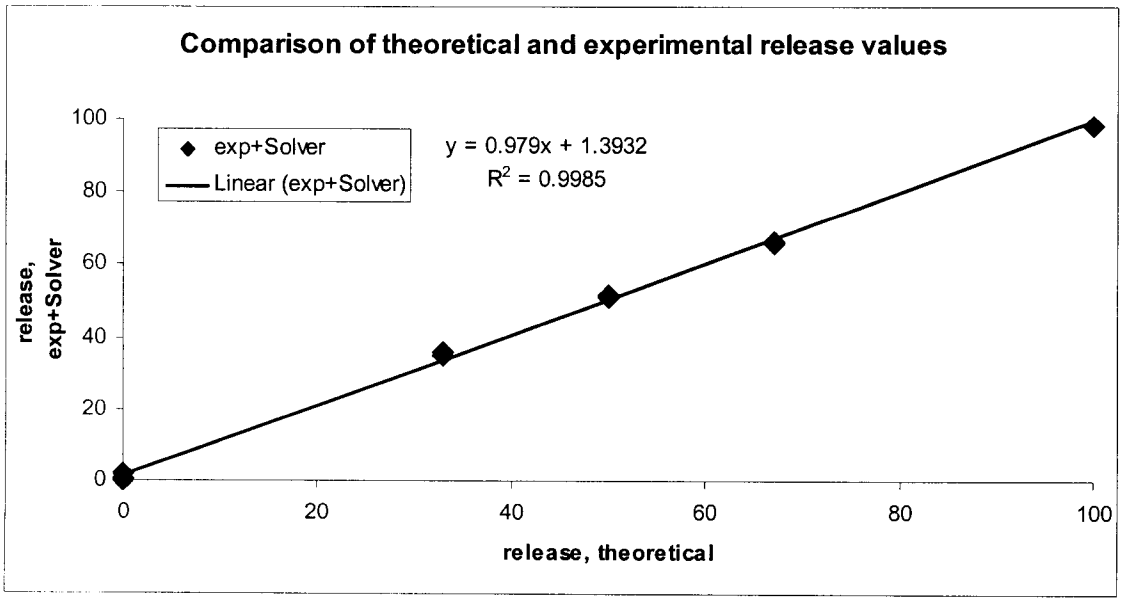
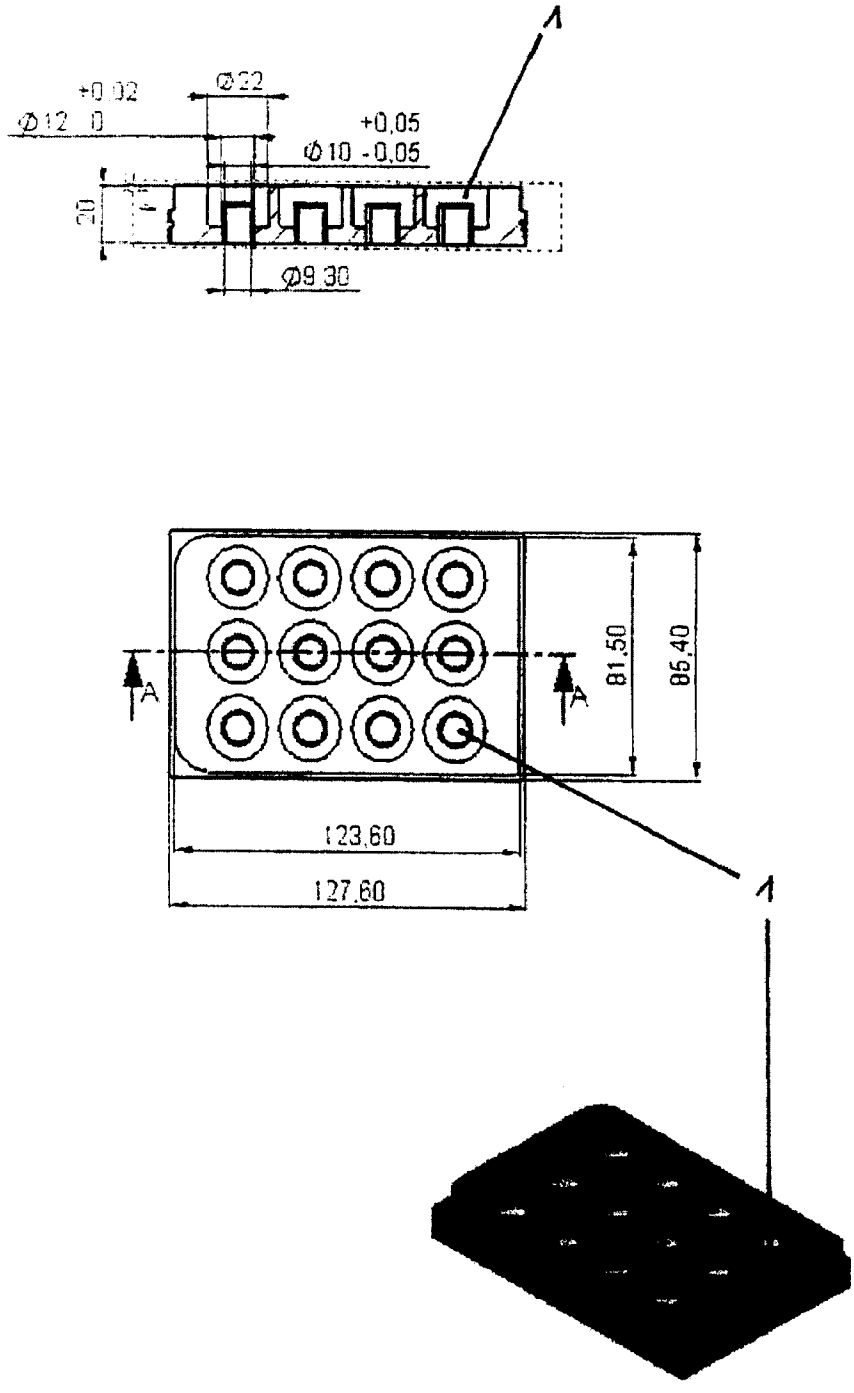


Figure 7: Special microtitre plate according to the invention for direct UV measurement



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/068682

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/15 G01N21/51 G01N21/25 B01L3/00 C12M1/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12M B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	page 18, line 25 - page 19, line 8 page 21, line 11 - line 20 figure 1	5,6
X	JP 59 116250 A (NOHIRA HIROYUKI) 5 July 1984 (1984-07-05)	2
Y	abstract	5
X	US 6 180 138 B1 (ENGH KEVIN R [US] ET AL) 30 January 2001 (2001-01-30)	3
Y	column 2, line 1 - column 4, line 9	6
X	WO 98/46981 A (LJL BIOSYSTEMS [US]) 22 October 1998 (1998-10-22) figure 7	8

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2007	21/02/2007

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/068682

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

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