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European Journal of Pharmaceutics and Biopharmaceutics 48 (1999) 225–232

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Dose uniformity and redispersibility of pharmaceutical suspensions I: quantification and mechanical modelling of human shaking behaviour

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Received 12 April 1999; received in revised form 7 May 1999; accepted 25 June 1999

Abstract

Precision and uniformity of single doses are required for most dosage forms including suspensions for oral and ophthalmic administration. Therefore, solids forming sediments or aggregates must be distributed homogeneously immediately before use. If settling occurs, leading pharmacopoeias require that suspensions be redispersible by shaking, but a standardised testing procedure for this property is not available. Obviously, such a test will have to be based upon observations of human shaking patterns. We report the results of a study in which human shaking acceleration profiles were measured and the shaking intensity characterised by the area under the frequency spectrum obtained by Fourier transform of the autocorrelation function. We also present a prototype of a mechanical redispersibility tester, which is more flexible and simulates human shaking behaviour more closely than its pneumatic forerunner, which was described earlier.

Keywords: Suspensions; Homogeneity; Dose uniformity; Shaking; Redispersibility; Fourier analysis

1. Introduction

Two factors determine the precision of each dose of a pharmaceutical suspension: the homogeneity of the dispersion and the volume removed for administration. The latter depends upon the viscosity of the fluid, the construction of the orifice and the measuring utensil and, last but not least, upon the dexterity of the user. Although the influence of formulation and package-related factors is difficult to estimate in practice, they are easily controlled during development by weighing. Homogeneity and redispersion after settling and aggregation are much more difficult to verify under conditions similar to those encountered in therapeutic use. In practice, suspensions are usually redispersed by shaking, but there are few studies indicating how long and intensively users shake pharmaceutical suspensions prior to administration, and which factors determine their shaking behaviour.

Pharmacopoeias require that pharmaceutical suspensions should be redispersible if they settle upon storage, but a standardised test for the dose uniformity of multiple dose suspensions after defined and reproducible shaking conditions is missing. The British Pharmacopoeia [1] states in the monograph on oral liquids under the subheading oral suspensions: 'Suspended solids may slowly separate on standing but are easily redispersed.' Similar requirements are given in the European Pharmacopoeia 1997 [2] and the United States Pharmacopoeia 23rd edition [3]. The problem is not addressed in the Pharmacopoeia of Japan XIIth edition [4] and in the International Pharmacopoeia 3rd edition [5], which do not contain suspension monographs.

In the first of this pair of publications, we present a mathematical and technical approach to the development of a realistic redispersion test procedure suitable for a pharmacopoeial monograph. Its application to commercial erythromycin ethyl succinate suspensions is discussed in the second, and a study on suspension eyedrops is forthcoming.

1.1. Shaking behaviour of humans

Apt [6] published a statistical study on the number of shaking cycles used by hospitalised patients to redisperse suspension eyedrops, but did not quantify the intensity. A qualitative assessment is due to Strobel [7], who studied the influence of shaking on the distribution of indomethacin in the eye. He described subtle shaking as 'containers were

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0939-6411/99/$ - see front matter
PII: S0939-6411(99)00045-4

turned upside-down twice' and vigorous shaking as 'containers were held between thumb and index finger and moved back and forth by rapid movements of the arm'. He found that the drug content of single drops was fairly constant after 20 vigorous strokes. Kwon et al. [8] quantified the intensity of shaking in healthy volunteers and hospitalised patients using the same acceleration sensor as we do. Her mathematical approach was similar, but she limited the time interval during which the acceleration signal was evaluated to 2.5 s. Besides, she used a dummy container, which was somewhat heavier than usual eye drop bottles. She found a significant correlation between shaking intensity and age and observed peak accelerations between 20 and 80 m/s^2. This corresponds approximately two to eight times the acceleration due to gravity at the earth surface. Schreiner et al. [9] used video recordings to assess the amplitude and velocity of shaking motions of subjects, who tried to speed up the dissolution of an acetylcholinesterase reactivating agent in double-chamber syringes.

1.2. Redispersibility assessment

Particles can be redispersed after settling or aggregation by motion relative to the fluid, which can be caused by oscillation of the container or by a rotating or oscillating stirrer within. Upon shaking, the forces acting on individual particles depend upon the mass of the particles and the difference of densities of the solid and the liquid. Most organic solids have densities in the range 1.5–1.8 g/cm^3, while the density of reasonably dilute aqueous solutions is close to 1 g/cm^3. The relative motion of particles or droplets also depends upon the magnitude of interparticulate forces and the viscosity of the fluid. The air bubble enclosed in the container may have a major effect upon redispersion because it has the greatest volume besides the fluid and because the difference between its density and that of the fluid is nearly 1 g/cm^3. No attempt has been made to model it quantitatively, since it behaves like a low-viscosity fluid, which disintegrates into minute droplets upon sufficiently vigorous shaking. The volume of air increases upon emptying. In some instances this seems to enhance the redispersion of particles attached loosely to the surface of a condensed sediment [8].

Over the past 40 years, a variety of methods has been used to assess the redispersibility of suspensions, but until very recently none has taken into consideration human shaking habits. Briner and Steiger-Trippi [10] used a 250 ml graduated cylinder for studying the redispersion of zinc oxide suspensions, which they tilted horizontally to an angle of $\pm 45°$ 30 times/min. These are probably unrealistically mild conditions, although the procedure is suited to rank formulations containing loose aggregates or sediments with respect to ease of redispersion. Matthews and Rhodes [11] studied griseofulvin suspensions by rotating bottles at a rate of 20 rev./min. Law et al. [12] used the same method to study the influence of positively charged liposomes on the stability of indomethacin suspensions. The redispersibility of erythromycin suspensions was assessed by Elkheshen [13], who counted the number of 180° inversions required for redispersion. Kwon [8] used a pneumatic shaker, which was comparable to the shaking intensity and peak acceleration of humans, but the acceleration profile was untypically rich in high frequency harmonics. Schreiner et al. [9] used a mechanical shaker similar to the one described below, in which both amplitude and frequency of the oscillations can be chosen within certain limits.

In the following sections the theoretical basis for quantification of human shaking behaviour is outlined and the method is applied to study the duration, amplitude, frequency as well as the resulting shaking intensity and power of agitation. Data were obtained from 79 subjects, who shook either a 5 ml polypropylene eyedrop bottle or a 100 ml glass bottle. Both vessels were filled with aqueous liquids and equipped with an acceleration sensor at the bottom. The results were compared with data from 31 subjects and patients studied by Kwon [8], who used the same sensor but a relatively heavy eyedrop-bottle dummy, and who did not account for the time of agitation. Subsequently, a mechanical shaker will be described, which could be used as a starting point for the development for a pharmacopoeial test.

2. Theory

Humans have vastly differing shaking habits ranging from mild rotatory movements of the hand joint to fast and wide movements of the whole arm, but most persons move primarily the forearm at the elbow joint. The resulting trajectories can be circular, curved or straight, either horizontal or vertical or at any angle with respect to the direction of gravity acceleration. It is not obvious that and how the shaking intensity can be uniformly quantified, irrespective of irrelevant details.

Fourier has shown that all periodic processes can be represented as superpositions of sine and cosine components in the time domain and as spectrum in the frequency domain [14]. This approach has been extended to include stationary irregular processes in autocorrelation analysis. Consider a sequence of observations of a fluctuating variable (in our case the motion, velocity or acceleration of a fluid container) taken at equal and sufficiently short intervals. The mean value and variance of the observations can be obtained by elementary computation, which need not be discussed here. An underlying periodicity of cycle length τ can be detected by autocorrelation analysis as follows [15]: let $h(t)$ be the time-dependent function describing the process under consideration and τ a variable lag time, then the empirical autocorrelation function $\rho(\tau)$ is for the continuous case

$$\rho(\tau) = \int_{-\infty}^{\infty} h(t)h(t+\tau)\mathrm{d}t \quad (1)$$

Its Fourier transform is

$$R(\omega) = \int_{-\infty}^{\infty} \rho(\tau)\mathrm{e}^{-\mathrm{i}\omega\tau}\mathrm{d}\tau = \int_{-\infty}^{\infty} h(t)\int_{-\infty}^{\infty} h(t+\tau)\mathrm{e}^{-\mathrm{i}\omega\tau}\mathrm{d}\tau\mathrm{d}t \quad (2)$$

Setting $x = t + \tau$, Eq. (2) can be rewritten as

$$\int_{-\infty}^{\infty} h(t)\mathrm{e}^{\mathrm{i}\omega t}\mathrm{d}t \int_{-\infty}^{\infty} h(x)\mathrm{e}^{-\mathrm{i}\omega x}\mathrm{d}x = H(-\mathrm{i}\omega)H(\mathrm{i}\omega) = |H(\omega)|^2 \quad (3)$$

where $H(\omega)$ denotes the Fourier transform of the time-dependent function $h(t)$.

This can be expressed as Parseval's theorem [14,16], which states that the total power of a signal is invariant under the transition from the time to the frequency domain:

$$P \equiv \int_{-\infty}^{\infty} h^2(t)\mathrm{d}t = \int_{-\infty}^{\infty} H^2(f)\mathrm{d}f \quad (4)$$

The invention of the Fast Fourier Transform Algorithm (FFT) by Cooley and Tukey [17], which is applicable to the analysis of functions sampled at constant intervals, was a major breakthrough in numerical mathematics and has greatly facilitated the analysis of time series. A detailed treatment is beyond the scope of this paper, interested readers are referred to monographs on the subject [14,18]. The dual view of signals in the time and frequency domains is used in a broad field of technical and other applications ranging from the design of electrical circuits and infrared spectroscopy to stock market analysis. For an outline of the method and applications see e.g. the review by Bracewell [19]. It has, however, rarely been applied to pharmaceutical problems. Exceptions are the analysis of mixing processes by Cartilier and Moes [20] and the quantification of cough intensity by Widdicombe [21].

The simplest form of oscillation is sinusoidal. Consider the distance of a body from its resting position as a function of time according to:

$$y = A\cos(\varphi + \omega t) \quad (5)$$

where the symbols have the following meaning: y elongation at time t (cm), A amplitude (maximal elongation) (cm), ω circular frequency (rad/s), ϑ phase shift (rad). We have

$$T = \frac{2\pi}{\omega} = \frac{1}{f} \quad (6)$$

with T cycle length (s), f frequency (Hz).

The standard example is a weight suspended on a spring. Neglecting damping effects, the weight oscillates sinusoidally if it is removed by an impulse from its equilibrium position in the gravity field. Under these idealised conditions, the kinetic energy of the weight in the neutral position is converted reversibly into positional energy associated with the compression or stretching of the spring at extreme elongations and back into kinetic energy. The kinetic energy is equal to

$$E_{\mathrm{kin}} = \frac{1}{2}mv^2 \quad (7)$$

The acceleration profile corresponds to the second derivative of the elongation, and we have

$$a(t) = \frac{\mathrm{d}^2y}{\mathrm{d}t^2} = -A\omega^2\cos(\varphi + \omega t) \quad (8)$$

Looking only at a stationary sinusoidal acceleration profile and disregarding the mass of the oscillating body, we can define the shaking intensity within one complete sinusoidal shaking cycle as a function of both amplitude and frequency:

$$I_{\mathrm{c}}(A,\omega) = \int_0^{2\pi/\omega} (-A\omega^2\cos(\varphi + \omega t))^2\mathrm{d}t \quad (9)$$

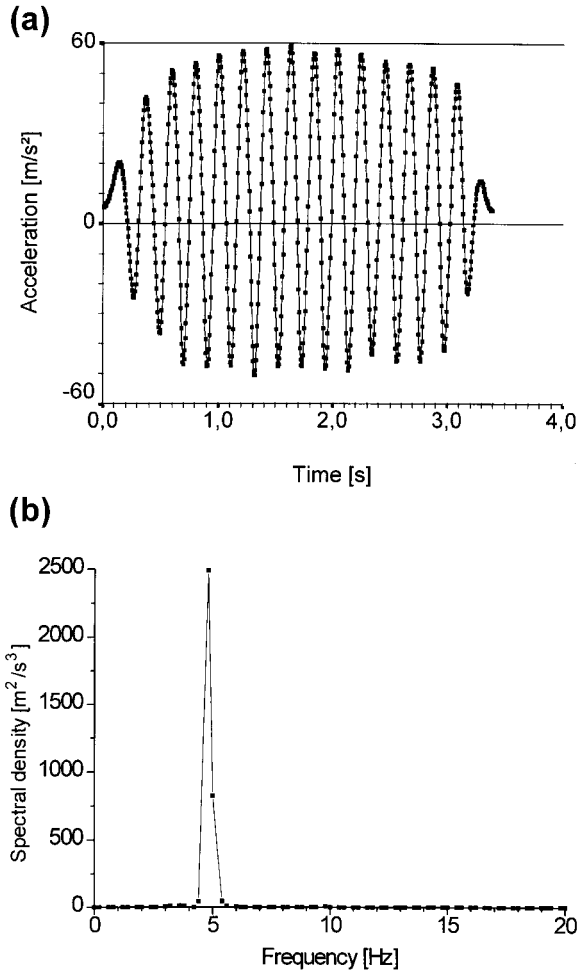

Fig. 1. Shaking profile of human subject (46 years, male), 100 ml bottle. (a) Acceleration profile. (b) Spectral density.

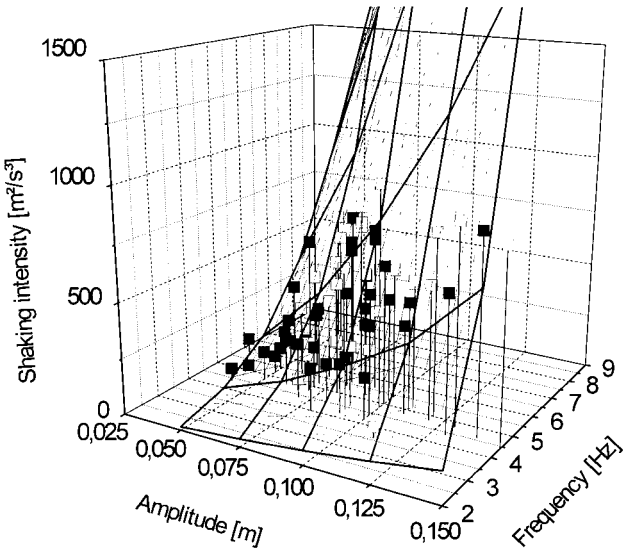

Fig. 2. Dependence of shaking intensity per cycle upon frequency and amplitude. Functional relationship for sinusoidal oscillations and observations. ■ subjects shaking 100 ml bottles; □ subjects shaking 5 ml bottles.

This approach can be generalised to include superpositions of a spectrum of functions with different frequencies.

2.1. Example

In Fig. 1a, the acceleration profile of one subject (male, 46 years) is given, Fig. 1b is the corresponding frequency spectrum. Note that it is nearly sinusoidal with only a narrow band of frequencies centred at 4.8 Hz. The amplitude of the stroke is 5 cm and for a body of 259 g weight we have a shaking energy per cycle of 56.7 W corresponding to an intensity per cycle of 219 m^2/s^3.

For the range of frequencies and amplitudes observed in subjects and patients, this relationship is plotted in Fig. 2, where the points represent observations.

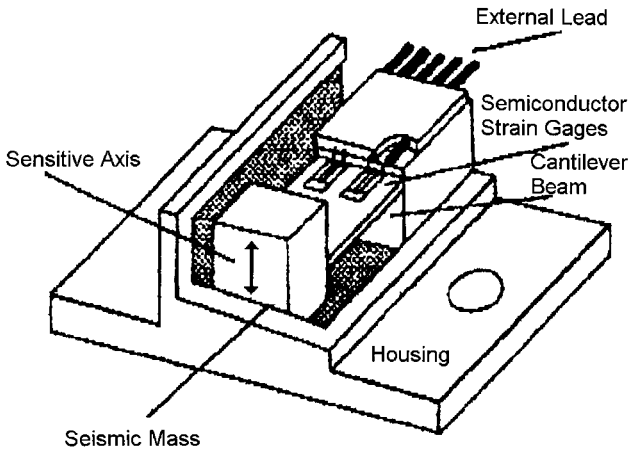

Fig. 3. Acceleration sensor (Entran EGAX-10 D).

3. Materials and methods

3.1. Shaking intensity of humans

Acceleration profiles of 47 men and 32 women were measured using an Entran EGAX-10 D sensor (Entran Sensoren, D-Ludwigshafen), in which deflections of a cantilever beam are converted to electrical signals (Fig. 3). Of

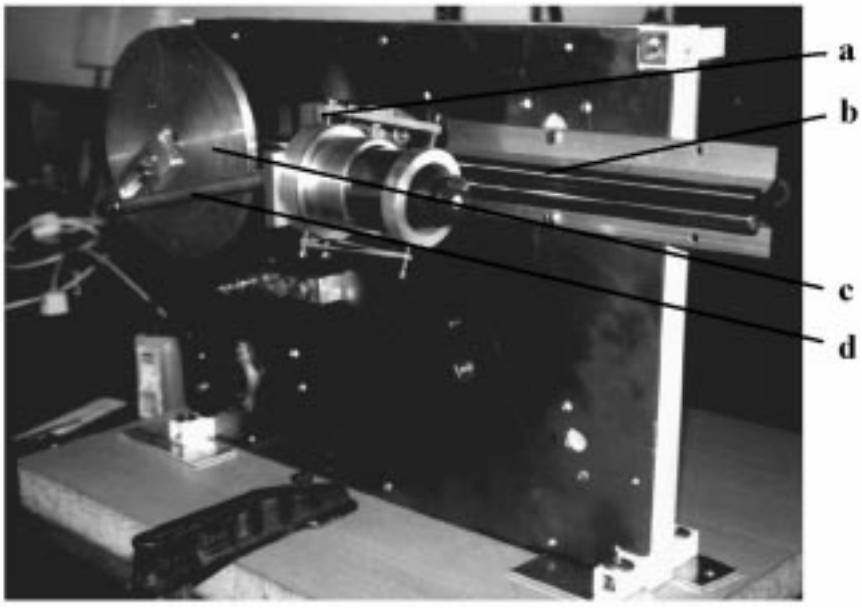

Fig. 4. Mechanical shaker. (a) carriage, (b) rail, (c) flywheel, (d) connecting rod.

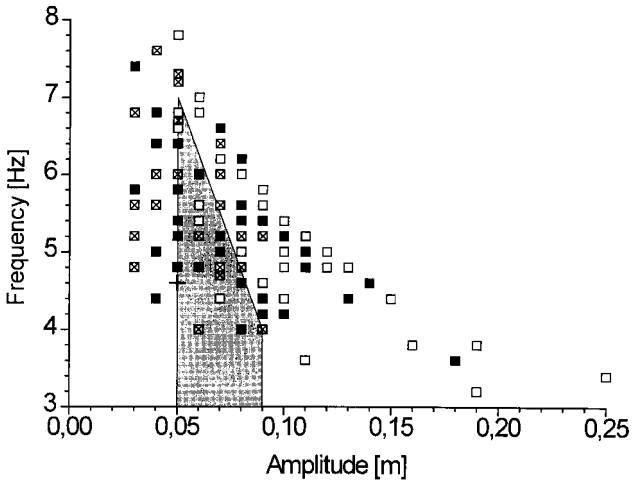

Fig. 5. Frequency and amplitude of human shaking patterns. Operating range and settings of mechanical shaker for testing erythromycin ethyl succinate suspensions. ■ 100 ml bottles; □ 5 ml bottles, open square with cross dummy container [5], shaded operating range of the mechanical shaker, + shaker settings for the redispersion experiment.

these subjects, 41 shook a glass bottle (length 13.5 cm, diameter 5 cm, weight 259 g) containing 65 g erythromycin ethyl succinate suspension, while 38 agitated a 5 ml polypropylene container (length 7.5 cm, diameter 2.5 cm, weight 34g) containing 3.5 g dexamethasone ophthalmic suspension. The sensor was attached to the bottoms of the vessels. Subjects were informed about the purpose of the study and instructed verbally and in writing to manipulate the objects exactly as they would handle medication prior to administration. Analogue signals were amplified and digitised at a sampling rate of 200 s^{-1} using a DAP 1200 signal processor (Microstar Labs, Redmont, WA, USA).

The duration of shaking and the maximum accelerations were measured directly. Some subjects had brief pauses interspersed in their shaking profiles, which were disregarded. The power spectrum in the frequency domain was computed by fast Fourier transform of the entire acceleration profile using the SPSS software package [22]. The main shaking frequency is the frequency component with the greatest spectral density.

The relationship between time and frequency domain has been discussed above for a continuous function *h(t)*. In our case the acceleration profiles were sampled at a rate of $N = 200\ s^{-1}$, i.e. we have a set of discrete measurements $h(t_j)$ at times t_j.

The discrete Fourier transform of N points h_k^2 in the time domain is the sum of N points H_n^2 in the frequency domain. In this case, the discrete form of Parseval's theorem [14,16] can be written as (cf. Eq. (4)):

$$\sum_{k=0}^{N-1} |h_k|^2 = \frac{1}{N} \sum_{n=0}^{N-1} |H_n|^2 \tag{10}$$

The area under the complete power spectrum corresponds to the total shaking intensity, see Figs. 1b, 8b and 9b. To standardise the intensity to one complete shaking stroke the total area was multiplied by the mean shaking period.

3.2. Mechanical shaker (Fig. 4)

Sample bottles are fastened in a horizontal position to a carriage (a) guided by a horizontal precision rail (b). The carriage is driven by a computer-controlled electric motor of 0.92 kW power consumption via a flywheel (c) and a connecting rod (d) with precision bearings.

The amplitude of the shaking movement can be set at 5, 7.5 and 9 cm by changing the radius of attachment to the flywheel. The frequency is controlled by preselecting the motor speed electrically using a thyristor circuit. The operating range of the shaker is indicated in Fig. 5, it covers an essential part of the central values of the amplitude-frequency area observed in humans. For the shaking experi-

Table 1
Shaking parameter statistics in subjects and settings of mechanical shaker

Population	Container	Quantiles	Shaking time (s)	Amplitude (m)		Frequency (s^{-1})	Intensity[a] (m^2/s^3)	Power[a] (W)
Subjects								
Age 18–73, female 16, male 25	100 ml	1st quartile	2.53	0.05[b]	0.04[c]	4.6	128	33.2
		median	3.47	0.08[b]	0.06[c]	5.2	292	75.6
Age 17–70, female 16, male 22	5 ml	1st quartile	1.66	0.07[b]	0.05[c]	4.7	302	10.3
		median	2.73	0.09[b]	0.07[c]	5.1	523	17.8
Age 21–50, female 21, male 12	Dummy[d]	1st quartile	n.a.	n.a.	0.04[c]	4.8	275	13.8
		median	n.a.	n.a.	0.06[c]	5.6	378	18.9
Patients								
Age 37–78, female 15, male 2	Dummy[d]	1st quartile	n.a.	n.a.	0.03[c]	4.6	54	2.7
		median	n.a.	n.a.	0.03[c]	5.0	95	4.8
Shaker	100 ml		3.0	0.05[b]	0.05[c]	4.2	134	34.7

[a] Per shaking cycle.
[b] Maximal observed amplitude.
[c] Mean amplitude computed from frequency and intensity assuming the shaking profile to be sinusoidal.
[d] Data from Kwon [22].

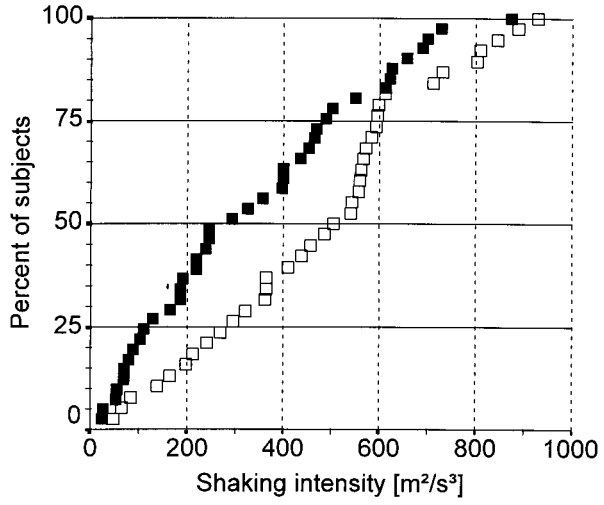

Fig. 6. Cumulative shaking intensity distributions in subjects. ■ 100 ml bottles (41 subjects); □ 5 ml bottles (38 subjects).

ments, the setting was chosen to match the first quartile of shaking intensities observed in subjects. For the redispersion experiments, the shaking time was 3 s, the frequency 4.2 Hz, corresponding to almost 13 strokes with an amplitude of 5 cm.

4. Results

Statistics of the shaking intensity of subjects for the two types or containers are summarised in Table 1. It was found that the shaking intensity is smaller for the heavier container (Fig. 6) and that it tends to decrease significantly (ANOVA, $P < 0.001$) with age (Fig. 7). This confirms observations by Kwon [8]. The amplitude of the light-weight eye-drop container was significantly greater than that of the heavier glass-bottle. There were no indications of a correlation between shaking intensity or amplitude or frequency and gender.

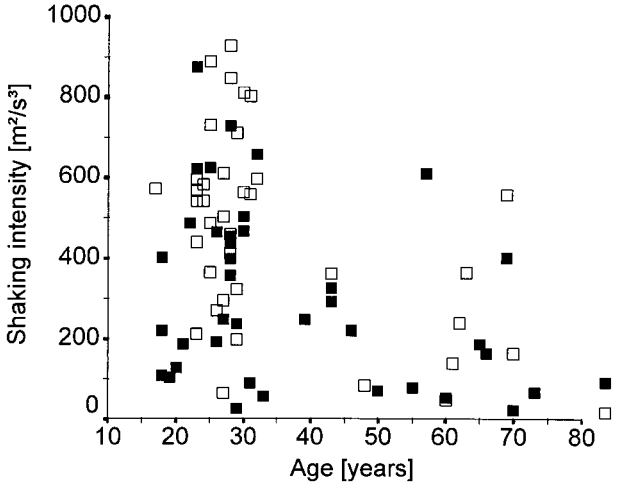

Fig. 7. Age dependence of shaking intensity in subjects. ■ 100 ml bottles; □ 5 ml bottles.

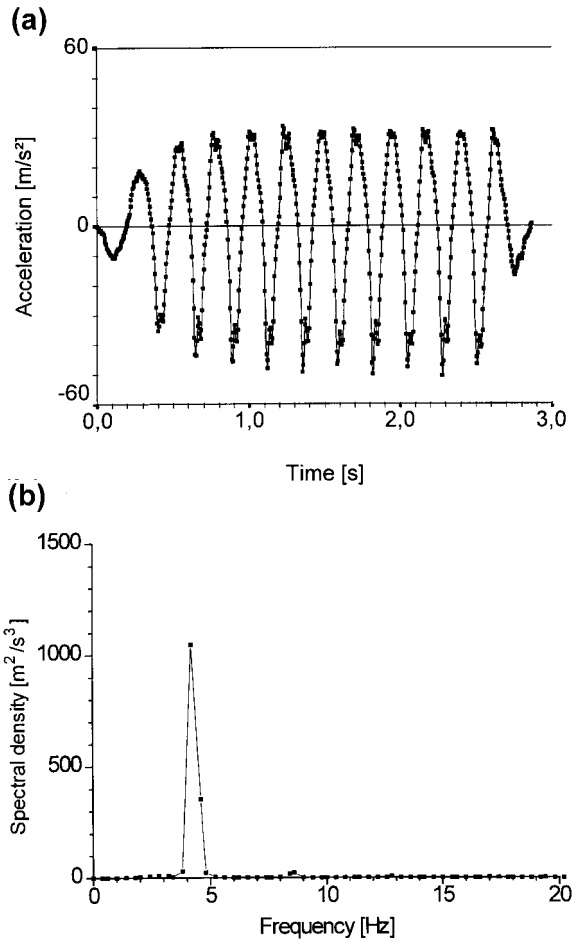

Fig. 8. Shaking profile of mechanical shaker. (a) Acceleration profile. (b) Spectral density.

The acceleration profile of the shaker is a reasonable approximation of the one observed in humans. In spite of significant efforts, irregularities at the points of maximal acceleration and deceleration could not be eliminated completely (Fig. 8a). The power spectrum (Fig. 8b) indicates, however, that the high-frequency components do not contribute significantly to the total shaking intensity. For the sake of comparison, the acceleration profile and the spectrum of Kwon's pneumatic shaker are given in Fig. 9. For testing marketed erythromycin suspensions, values for the amplitude and the frequency of the shaker were selected so that the intensity per cycle corresponded to that of the first quartile of the subjects.

5. Discussion

Some pharmaceutical suspensions are subject to problems of dose uniformity due to physical instability. Pharmacopoeias require that suspensions should be redis-

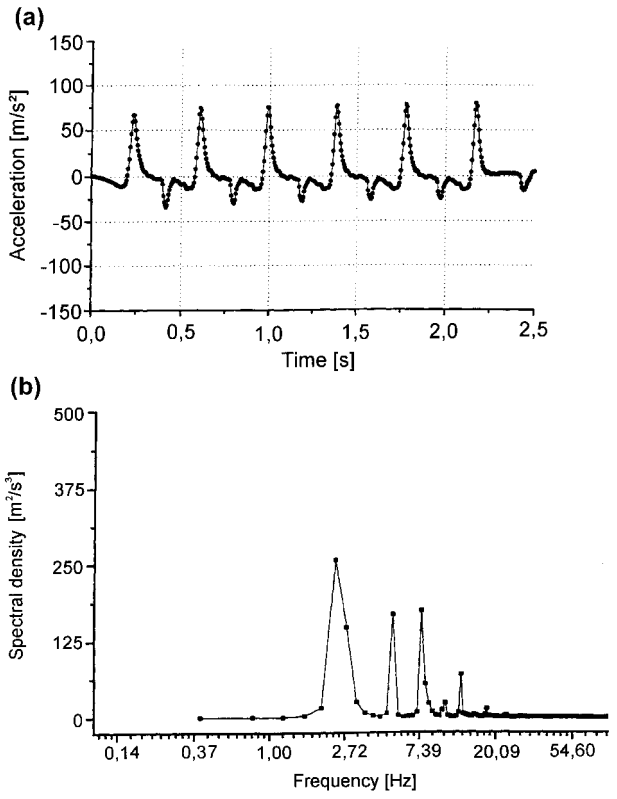

Fig. 9. Shaking profile of pneumatic shaker. (a) Acceleration profile. (b) Spectral density. In Kwon's publications [8,23] the ordinate was scaled differently because the spectral density was not divided by the sampling rate (cf. Eq. (10)).

persible, but a standardised test for this property is missing. Obviously, such a test should be based on the performance that can be expected from the target population, and clear instructions should be given to users. It has been shown that in one suspension eyedrop preparation, approximately 75% of the active ingredient cannot be removed from the container by shaking because it adheres tenaciously to the bottom of the container [6]. Some manufacturers are aware of the problem of incomplete redispersion, and a mechanical shaking intensity indicator is available for Chibro Amuno® 3 eyedrops (Chibret, D-Munich). It may be noted that increasing the duration of agitation does not improve the homogeneity of a partially caked suspension if the intensity is too low to break particles away from the surface of the condensed sediment. The mechanical shaker presented here is one component of a computer-controlled automatic suspension tester, which includes also components for automatic sampling. More sophisticated devices based on linear electromagnetic drives have been conceived and will be tested in the future.

For any testing device, reasonable operating conditions have to be fixed. It appears sensible to take relevant characteristics of the target population into account. Critical limits for the duration and intensity of shaking may have to be relatively low for products like suspension eye drops, which are intended for use by older patients without assistance, while for paediatric preparations, where mothers, nurses or other younger persons assist in the administration, more vigorous and longer agitation can be expected. Adjusting the shaker to the 25th percentile of the shaking intensity distribution of the target population may be a reasonable starting point. In any case, more shaking studies will be necessary to obtain reliable estimates of empirical intensity distributions and their covariates in distinguishable subpopulations.

6. Conclusions

Although the market segment of pharmaceutical suspensions is relatively small, some of them are used for the management of severe diseases with potent drugs, where exact dosing is essential. Therefore it is felt, that an apparatus similar to the one presented here should be developed for a pharmacopoeial test of their redispersibility.

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