The Effect of Cross-Linking on the *In Vivo* Disintegration of Hard Gelatin Capsules

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Purpose. To evaluate if the cross-linking of gelatin affects *in vivo* capsule disintegration.

Methods. Scintigraphic investigation in nine healthy volunteers to provide for a real time visualisation of capsule disintegration.

Results. The moderately stressed capsules failed the USP dissolution specification for acetaminophen capsule when tested in water and conventional SGF but passed with the addition of pepsin. Moderately stressed capsules started to disintegrate at 10 ± 6 minutes (range 6 to 24 minutes) compared to 8 ± 2 minutes (range 5 to 11 minutes) for the unstressed capsule.

Conclusions. The results of the study clearly demonstrate that with the incisive technique of gamma scintigraphy there are no differences in the *in vivo* disintegration properties of moderately stressed and unstressed capsules.

KEY WORDS: hard gelatin capsule; cross-linking; disintegration; scintigraphy; gastrointestinal tract.

INTRODUCTION

The hard gelatin capsule has been used for many years to deliver drugs in the form of powders, granules, pellets and tablets to the gastrointestinal tract. The major component of the capsule is gelatin which has the ability to form highly suitable films, capable of dissolving readily in the stomach. It has been reported that certain conditions, especially the existence of trace amounts of aldehydes in the capsule fill material or hot and humid storage, can render gelatin partially insoluble in water due to a cross-linking reaction (1,2). The insolubilization of gelatin in water with consequently reduced *in vitro* dissolution rate has been shown to have no *in vivo* significance (3).

However, to investigate the *in vivo* relevance of gelatin cross-linking in more detail a gelatin capsule working group comprising FDA and industry representatives was formed. Over the last four years the group has been carrying out a program of research to develop gelatin capsules with well characterized degrees of cross-linking so as to study the effect of cross-linking on both *in vitro* dissolution and bioavailability of a model drug product (4).

Stressed hard gelatin capsules have been prepared by exposing capsule shells to formaldehyde laced lactose (5). Capsules stressed to the fail/pass level failed to meet the USP dissolution specification for acetaminophen capsules when tested in water and simulated gastric fluid (SGF) without pepsin. However, in the acidic environment of the stomach the capsules would also be exposed to the enzyme pepsin. When pepsin was added to the SGF the capsules passed the USP dissolution specification. Capsules stressed to the fail/fail level failed to meet the USP dissolution specification for acetaminophen capsules when tested in water and SGF without pepsin and also failed when tested in the dissolution medium containing pepsin. In a bioequivalence study with these acetaminophen capsules, the moderately stressed or fail/pass capsules were found to be bioequivalent to the unstressed capsules whereas the severely stressed or fail/fail capsules were bioinequivalent to the unstressed capsules (6). Therefore the concept of using enzyme in the dissolution medium when a hard gelatin capsule fails dissolution in water or acid due to cross-linking of the capsule shell is now becoming accepted (7).

Conventional pharmacokinetic studies can be viewed as a 'blunt' instrument for comparing the Fail/Pass (moderately stressed) and Pass/Pass (unstressed) capsules and whilst formal bioequivalence is reassuring, it provides no real data on the potential for *in vivo* pellicle formation. In addition, it has been suggested that for drugs with a narrow "window for absorption" the issue of *in vivo* pellicle formation may have a more significant effect and could have bioequivalence implications. The non-invasive imaging technique of gamma scintigraphy allows us to assess the *in vivo* behaviour of the gelatin capsule shell regardless of its contents, as it provides a real-time visualisation of the actual release process (8,9). In this paper, we report on the use of scintigraphy to validate the scientific basis of the two tier dissolution process and to add "value" to the previous pharmacokinetic evaluation (6). The objective of this study was therefore to evaluate if cross-linking of gelatin really affects *in vivo* capsule disintegration.

MATERIALS AND METHOD

Capsule Manufacture and Testing

A method of stressing hard gelatin capsule shells was developed (5). The degree of stressing or cross-linking was monitored indirectly by measuring the dissolution of acetaminophen using the USP method II at 50 rpm. The procedure involved exposure of lactose powder to formaldehyde vapour followed by an accurate measurement of aldehyde contamination. Dilution of samples from the treated powder with pure lactose gave the desired levels of formaldehyde contamination. Lactose containing 20 ppm formaldehyde was filled by hand into size 1 natural transparent capsules (Capsugel Greenwood SC Lot No. 515736) which were stored for six days at room temperature. The capsules were then emptied and along with the unstressed capsules filled by hand with 280 mg acetaminophen and 2 mg of samarium oxide. Subsequent irradiation in a neutron source converted the non-radioactive tracer (^{152}Sm) into a gamma emitting radioisotope (^{153}Sm) (9). Prior to the clinical phase of the project, trial irradiations were undertaken

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which demonstrated that the release characteristics of the preparations were not affected by exposure to the neutron flux.

In vitro release characteristics of the capsules were assessed using the two tier dissolution strategy. The dissolution media included water and SGF with and without pepsin. Pepsin with an activity level of 800–2500 units/mg protein (1:10,000) and a concentration of 3.2 g/l was used. The unstressed capsule met the USP dissolution specification for acetaminophen capsules when tested in water and SGF without pepsin (Pass/Pass) (Table 1). The moderate stressing condition resulted in capsules which failed the USP dissolution specification for acetaminophen capsules when tested in water and SGF without pepsin but which met the USP dissolution specification when tested in SGF with pepsin (Fail/Pass) (Table 1).

Clinical Trial Design

This was a single, blind, randomised crossover study in nine healthy male or non-pregnant female volunteers. Each volunteer was examined by a physician before the study and was judged to be in good health on the basis of medical history, physical examination and routine laboratory data. The clinical protocol was approved by an independent Ethics Committee and approval for administration of radiolabelled preparations was obtained from the Department of Health, London. Each subject provided written informed consent to participate in the study.

Study Protocol

The volunteers arrived fasted (from midnight) at the study site. Anterior anatomical markers containing 0.1 MBq ^{99m}Tc were taped to the skin over the right lobe of the liver, in the same transverse plane as the distal end of the oesophagus for each subject. In line with the study randomisation, each subject received either the Fail/Pass or Pass/Pass capsule with 200 ml of water.

Anterior scintigraphic images, each of 50 seconds duration, were taken using a gamma camera (General Electric Maxicamera) with a 40 cm field of view and fitted with a low energy parallel hole collimator. Images were recorded every minute until complete capsule disintegration was observed and were acquired with the subjects standing in front of the gamma camera. The images were recorded using a Bartec computer system and were stored on optical disk for subsequent analysis.

Table 1. Dissolution[a] of Acetaminophen from Pass/Pass (Unstressed) and Fail/Pass (Moderately Stressed) Capsules

	Average percent released at 45 min (range)[b]		
Capsule Type	Water	SGF without pepsin	SGF with pepsin[c]
Pass/Pass	97 (94–99)	97 (94–100)	N/A[d]
Fail/Pass	61 (53–69)	53 (33–68)	94 (87–100)

[a] USP Paddle apparatus, 50 rpm, 900 ml, 37°C.
[b] N = 6.
[c] 3.2 g/l, Activity 1: 10,000 Product No. P7,000, Sigma Chemical Company.
[d] Not applicable.

Table 2. Disintegration Profile of Acetaminophen Filled Hard Gelatin Capsules (Fail/Pass (Moderately Stressed) Capsules)

		Complete capsule disintegration	
Subject number	Initial capsule disintegration	(minutes post-dose)	(minutes post-ICD)
1	7	11	4
2	9	11	2
3	9	16	7
4	5	9	4
5	24	26	2
6	11	22	11
7	8	11	3
8	8	10	2
9	6	18	12
Mean	10	15	5
SD	6	6	4
Median	8	11	4
n =	9	9	9

Note: ICD = initial capsule disintegration.

The dynamic imaging of *in vivo* capsule performance provided an incisive assessment of the disintegration process. Initial capsule disintegration was defined as the time taken to detect signs of release of radioactive marker from the capsule, whilst complete disintegration was defined as the time at which all the radiolabel had dispersed within the gastrointestinal tract and no signs of a distinct 'core' remained.

RESULTS AND DISCUSSION

In vivo disintegration data are provided for the Fail/Pass and Pass/Pass capsules in Tables 2 and 3, respectively. Scintigraphic images showing the key stages of capsule disintegration in a representative subject are highlighted in Figure 1.

Table 3. Disintegration Profile of Acetaminophen Filled Hard Gelatin Capsules (Pass/Pass (Unstressed) Capsules)

		Complete capsule disintegration	
Subject number	Initial capsule disintegration	(minutes post-dose)	(minutes post-ICD)
1	8	13	5
2	5	10	5
3	5	5	0
4	7	12	5
5	10	12	2
6	11	15	4
7	10	12	2
8	9	12	3
9	6	15	9
Mean	8	12	4
SD	2	3	3
Median	8	12	4
n =	9	9	9

Note: ICD = initial capsule disintegration.

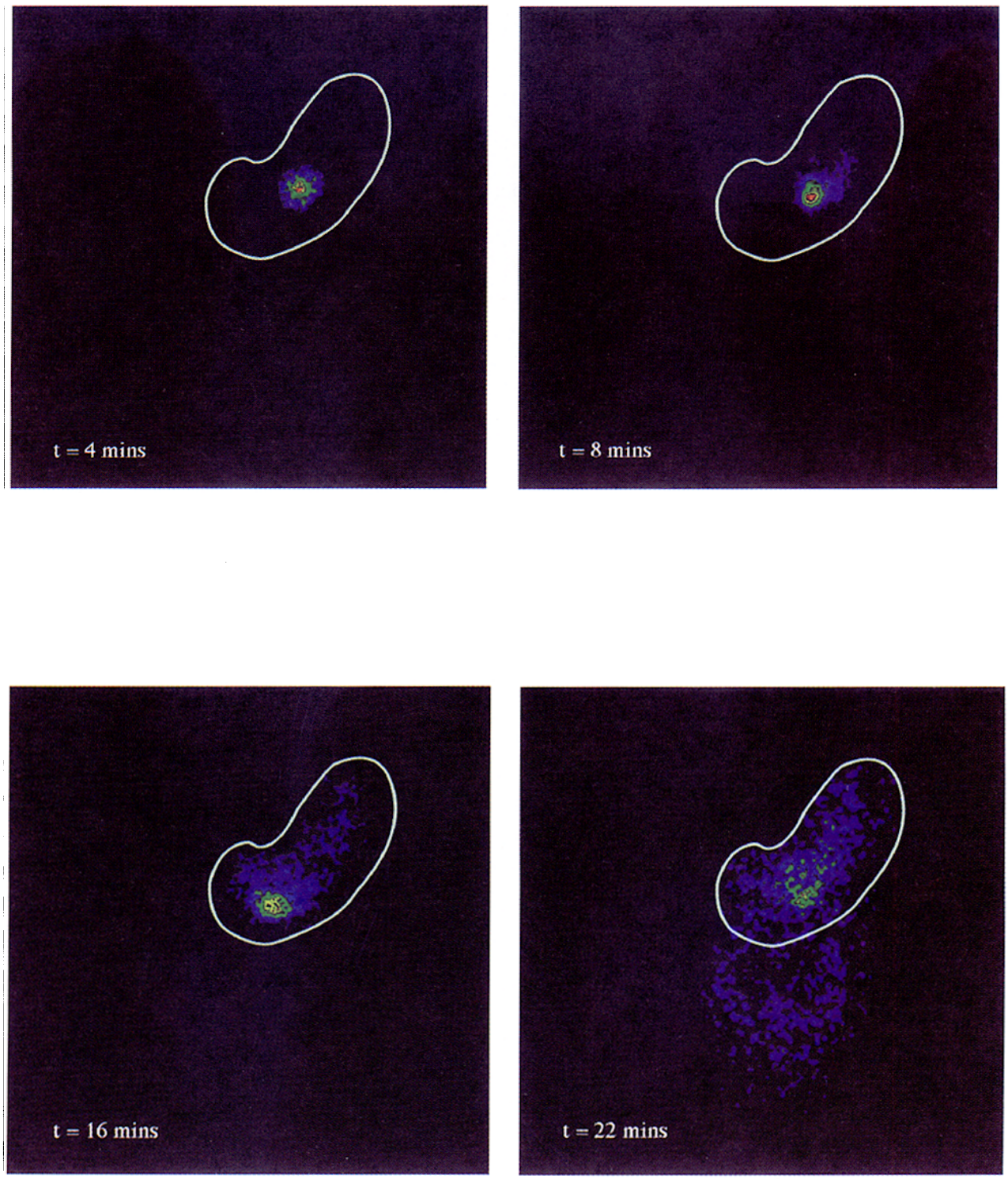

Fig. 1. Scintigraphic images of *in vivo* capsule disintegration in subject 9; (a) Fail/Pass capsules and (b) Pass/Pass capsules.

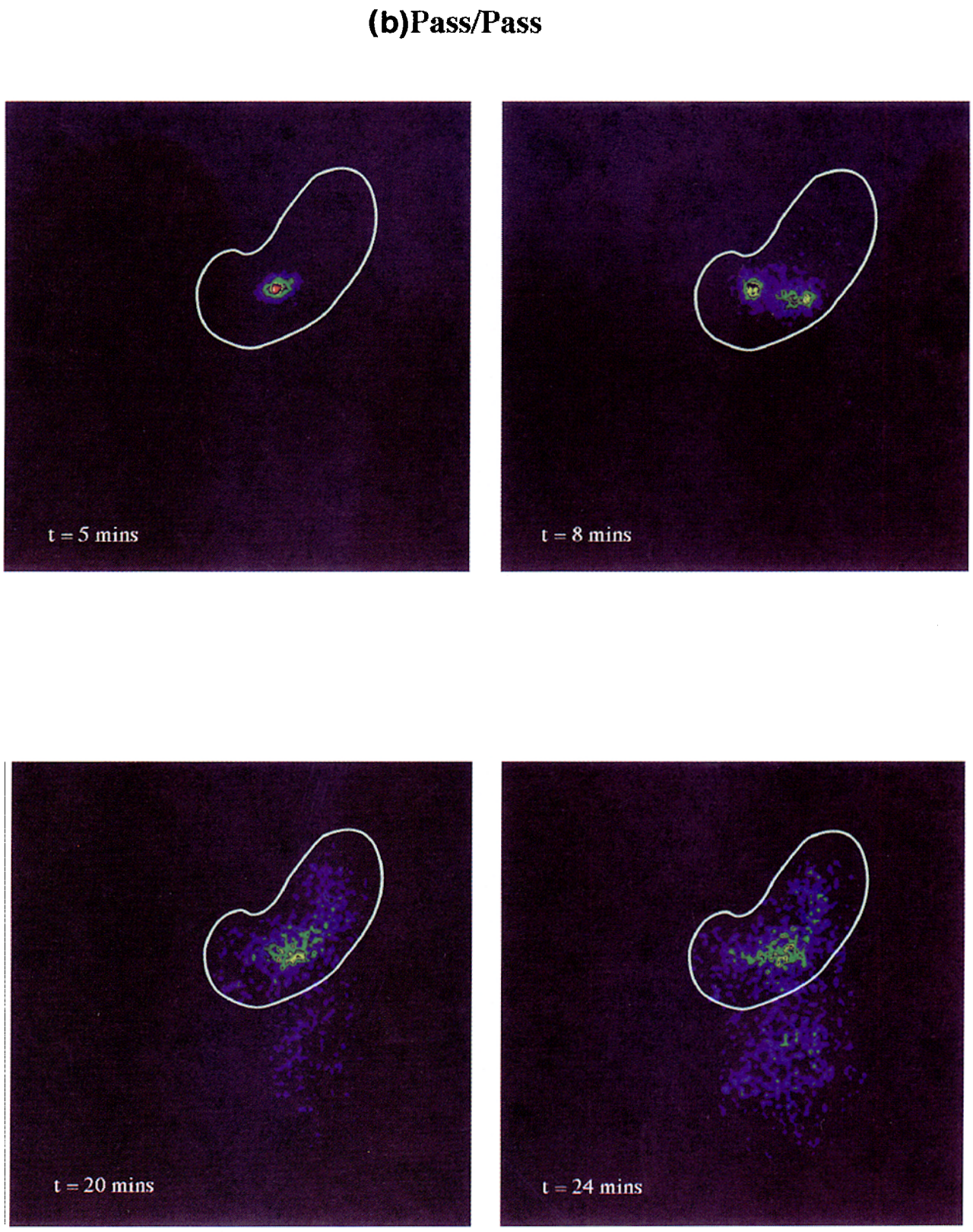

Fig. 1. *Continued.*

The discriminatory ability of the two-tier dissolution strategy is clearly observed from a comparison of the *in vitro* release dissolution data provided in Table 1. The release of acetaminophen from the moderately cross-linked capsule in SGF (without pepsin) is significantly lower than the unstressed control. By the addition of pepsin, the *in vitro* dissolution process is enhanced and the stressed capsule meets the specification.

The onset of *in vivo* capsule disintegration for the unstressed capsule was 8 ± 2 minutes (range 5 to 11 minutes) and complete disintegration occurred at 12 ± 3 minutes (range 5 to 15 minutes). Once initial disintegration of the capsule was observed, complete release of the contents followed in 4 ± 3 minutes (range 0 to 9 minutes). Interestingly, there is a lack of good scintigraphic research evaluating the *in vivo* disintegration of unstressed capsules; the majority of previous studies have had an imaging frequency of circa every 10 minutes and have not precisely evaluated capsule disintegration. However, the findings are in good accord with the "ballpark" disintegration data acquired in other scintigraphic studies (10–12).

Initial *in vivo* disintegration of the moderately stressed capsule occurred at 10 ± 6 minutes (range 5 to 24 minutes) and complete release of the capsule was observed at 15 ± 6 minutes (range 9 to 26 minutes). The time taken for complete capsule disintegration once the process had commenced was again rapid; 5 ± 4 minutes (2 to 12 minutes).

The results of the study clearly demonstrate that with the incisive technique of gamma scintigraphy there are no differences in the *in vivo* disintegration properties of moderately stressed and unstressed capsules. It is therefore clear that *in vitro* pellicle formation in traditional USP dissolution testing is not replicated in the human gastrointestinal tract and that the utilisation of a two-tier dissolution strategy using the gastric enzyme is entirely justified. It should also help to allay concern that the absorption of drugs with a narrow window of absorption would be adversely influenced by a moderate level of cross-linking in gelatin capsules.

REFERENCES

1. G. A. Digenis, T. B. Gold, and V. P. Shah. Cross-linking of gelatin capsules and its relevance to their *in vitro-in vivo* performance. *J. Pharm. Sci.* **83**:915–921 (1994).
2. D. Cadé, N. Madit, and E. T. Cole. Development of a test procedure to consistently cross-link hard gelatin capsules with formaldehyde. *Pharm. Res.* **11**:5–147 (1994).
3. M. Dey, R. Enever, M. Kraml, D. G. Prue, D. Smith, and R. Weierstall. The Dissolution and bioavailability of etodolac from capsules exposed to conditions of high relative humidity and temperatures. *Pharm. Res.* **10**:1295–1300 (1993).
4. H. Malinowski. Overview of the process: Issues, Research and Outcome. AAPS: "Hard and Soft Gelatin Capsules: Issues, Research and Outcome" Boston (1997).
5. E. T. Colé, N. Madit, and D. Cadé. Method of stressing hard gelatin capsules. AAPS: "Hard and soft gelatin capsules: Issues, Research and Outcome" Boston (1997).
6. R. M. Mhatre, H. Malinowski, H. Nguyen, M. C. Meyer, A. B. Straughn, L. Lekso, and R. L. Williams. The effects of cross linking in gelatin capsules on the bioequivalence of acetaminophen. *Pharm Res.* **14**(11):S-251 (1997).
7. V. A. Gray. Two-tier dissolution testing. AAPS: "Hard and soft gelatin capsules: issues, research and outcome" Boston (1997).
8. I. R. Wilding, A. J. Coupe, and S. S. Davis. The role of gamma scintigraphy in oral drug delivery. *Adv. Drug Del. Rev.* **7**:87–117 (1991).
9. S. P. Newman and I. R. Wilding. The importance of gamma scintigraphy in pharmaceutical development. *J. Pharm. Med.* **6**:21–33 (1996).
10. J. A. Ganley, J. McEwan, R. T. Calvert, and M. C. J. Barker. The effect of *in vivo* dispersion and gastric emptying on glibenclamide absorption from a novel, rapidly dissolving capsule formulation. *J. Pharm. Pharmacol.* **36**:734–739 (1984).
11. D. Y. Graham, J. L. Smith, and A. A Bouvet. What happens to tablets and capsules in the stomach: Endoscopic comparison of disintegration and dispersion characteristics of two microencapsulated potassium formulations. *J. Pharm. Sci.* **79**:420–424 (1990).
12. C. J. Kenyon, F. Stewart, G. McClelland, and I. R. Wilding. The use of pharmacoscintigraphy to elucidate food effects observed with a novel proteinase inhibitor (Saquinavir). *Pharm. Res.* **15**:415–420 (1998).