high and variable because of the interferences of some naturally occurring amines in urine. Therefore, some modifications of the method are necessary. This subject is now under investigation.

Experimental

1. Apparatus and chemicals

Maprotiline hydrochloride of pharmaceutical grade was used. The other reagents were of analytical grade. Absorbance measurements were made with a Shimadzu UV-160 A UV visible spectrophotometer.

2. Solutions

The stock solution of **1** (1.27×10^{-4} M) was prepared in water. The solution of **2** (6.4×10^{-4} M) was prepared in phosphate buffer solution of pH 6.0. A solution of 20% **3** in methanol was used.

3. Procedure

3.1.1. Preparation of calibration graphs

Suitable aliquots of the stock solution of **1** (0.25–2 ml) were transferred to stoppered glass tubes and 1 ml of **2**-solution and 3 ml of phosphate buffer (pH 6.0) were added. The volume was brought to 5 ml with phosphate buffer. The mixture was extracted with 5 ml of dichloromethane for 2 min with a vortex mixer. The absorbance of the dichloromethane layer was measured at 409 nm against a blank solution prepared similarly.

3.1.2. Preparation of calibration graphs using TBAH

Suitable aliquots of the stock solution of **1** (0.50–1.75 ml) were transferred to stoppered glass tubes and 1 ml of **2**-solution and 3 ml of phosphate buffer (pH 6.0) were added. The volume was brought to 5 ml with phosphate buffer. The mixture was extracted with 10 ml of dichloromethane for 2 min with vortex mixer. To 3 ml of the solution, 25 µl of **3**-solution were added. The absorbance of the dichloromethane layer was measured at 630 nm against a blank solution prepared similarly.

3.1.3. Assay procedure for tablets

Tablet powder equivalent to one tablet (each tablet contains 25 mg of **1**) was accurately weighed and transferred into a 500 ml calibrated flask. Water (200 ml) was added, then the mixture was shaken mechanically for 20 min and diluted with water to the volume, mixed and filtered. The filtrate (1 ml) was analysed similarly as described in section 3.1.1. beginning with "1 ml of **2**-solution". The amount of **1** in tablets was calculated from the regression equation obtained from the calibration graph.

Acknowledgements: This work was supported by the Research Fund of the University of Istanbul, Project number: T-263/260696.

References

1 Soonhee,K. S.; James, B. S.; in: Florey, K. (ed.): Analytical Profiles of Drug Substances, Vol. 15, p. 422, Academic Press, New York 1986
2 Öztunç, A.: Mar. Üniv. Ecz. Derg. **5**, 139 (1989)
3 Ersoy, L.; Alpertunga, B.: Analyst **113**, 1745 (1988)
4 Kotova; Lyubov, A.: From Izobreteniya **47**, 159 (1992)
5 Prinoth, M.; Mutschler, E.: J. Chromatogr. **305**, 508 (1984)
6 Moehrke, W.; Mutschler, E.; Spahn, H.; Weber, H.: Appl. 19 Aug. (1983) 29 pp
7 Drebit, R.; Baker, G. B.; Dewhurst, W. G.: J. Chromatogr. **432**, 334 (1988)
8 Maurer, H.; Pfleger, K.: J. Chromatogr. **305**, 309 (1980)
9 Weingaertner, K.; Wallenstein, F. A.: Labor-Med. **11**, 267 (1988)
10 Robinson, K.; Smith, R. N.: J. Immunoassay **6**, 11 (1985)
11 The United States Pharmacopoeia, Twenty-third Revision (USP XXIII), p. 931 United States Pharmacopoeial Convention, Rockville 1995

Received September 22, 1999
Accepted December 1, 1999

Prof. Dr. Aysel Öztunç
Department of Analytical Chemistry
Faculty of Pharmacy
University of Istanbul
34452 Istanbul
Turkey

Laboratoire d'activation moléculaire[1], Université Mohamed Premier, Faculté des Sciences, Oujda, Maroc, and Laboratoire de Pharmacochimie et Biopharmacie[2], Université de Haute Normandie, Rouen, France

Preparation and evaluation of benzyl benzoate-loaded poly-ε-caprolactone nanoparticles

M. Berrabah[1], D. Andre[2], P. Verite[2], A. Zahidi[1] and O. Lafont[2]

Aqueous suspensions of polymeric nanoparticles have been used for pharmaceutical applications such as drug targeting [1, 2]. They can be produced either by polymerisation [3, 4] or by dispersion of preformed polymers [5, 6]. The main difficulty encountered in the colloidal suspension systems was the separation of the nanoparticles from the liquid medium. This paper describes two methods to separate nanoparticles from the aqueous phase, namely ultracentrifugation and flocculation [7]. The later technique was performed in the presence of electrolytes and gave similar results. The determination of benzyl benzoate (BB) in nanoparticles and in the supernatant can be performed without derivatization by GC-MS [8].

BB determinations were performed simultaneously in the aqueous phase and in the sediment, on aliquots of the same sample of nanoparticle suspension at a concentration of 5 µl BB per ml suspension. When 10 ml of a nanoparticle suspension were ultracentrifuges at $50{,}000 \times g$ for 45 min, a very low concentration (1,39%) of BB was found in the aqueous phase, and 98,61% of the BB was entrapped in the polymer particles (encapsulated). When a sample of nanoparticles (10 ml) was flocculated directly by addition of electrolytes, the percentage of free BB was 1.16%, against 98.84% BB associated with the polymer. This result was expected because BB is a lipophilic compound which is not soluble in water. When the formulations were tested for release *in vitro* at pH = 7.4 (Fig.), it was found that BB and phenobarbitone were released in a biphasic pattern, characterized by an initial and variable rapid release period followed by a continuous and much slower release phase. The best ratio between free and encapsulated BB was obtained in the case of 4 ml BB/100 ml suspension. Ten ml of the nanoparticle suspension were flocculated. For this sample four successive determinations allowed calculation of the inter-assay precision (RSD). The results were as follows: 0.363 µl BB per ml

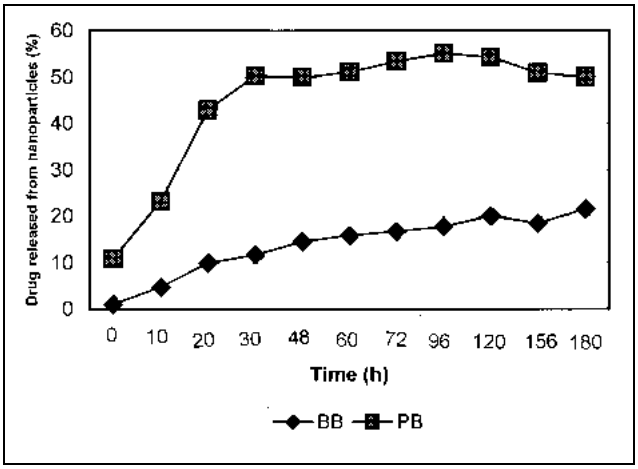

Fig.: *In vitro* release profile of benzyl benzoate (BB) and phenobarbitone (PB) from nanoparticle suspensions

suspension (RSD 2,4%) in the aqueous phase; 35.255 µl BB per ml of suspension (RSD 3.7%) associated.
In conclusion, ultracentrifugation was used to separate the polymer particles from the aqueous phase, but the best method to determine free, encapsulated and released BB was flocculation. The nanoparticle suspensions were destabilized by addition of electrolytes. The presence of electrolytes obviated the addition of surfactant or polymeric colloids for stabilization. Flocculation reduces the time of analysis and avoids potential adsorption of the drug onto the polymer. It can easily be adopted to evaluate drug entrapped into nanoparticle suspensions and to estimate drug release from nanoparticles.

Experimental

1. Apparatus

A Hewlett-Packard 7673A liquid autosampler, operated in the fast mode for splitless injection, was used. A Hewlett-Packard fused-silica capillary column (25 m × 0.22 mm i.d., 0.11 m film thickness) coated with cross-linked 5% phenylmethyl-silicone was used. A Hewlett-Packard 5970A mass spectrometer, operated in the the electron-impact mode, was directly interfaced with a 5890 gas chromatograph by the capillary column. Ultracentrifugation was performed with a Beckman L8-55 ultracenrifuge (Beckman Instruments, Berkeley, CA, USA), equipped with a 40 TR rotor. $CuSO_4 \cdot 5\ H_2O$ (Aldrich-Chemie, Steinheim, Germany) was lowly added to the nanoparticle suspensions under mechanical stirring.

2. Materials

Poly-ε-caprolactone (PεC), benzyl benzote (BB), benzyl alcohol (BA), and phenobarbitone (PB) were purchased from Aldrich-Chemie (Steinheim, Germany), Prolabo (Paris, France) or Rhone-Poulenc-Rorer (Paris, France). The chemicals used were high grade analytical preparations.

3. Chromatographic conditions

The initial oven temperature was maintained at 70 °C for 1 min, and then alowed to rise at a rate of 10 °C per min up to 230 °C for 3 min. Injector and transfer line were maintained at 240 °C during the experiment. The chromatogram obtained after injection of BB and BA in full scan-mode showed two peaks. Corresponding MS show that the following ions should be monitored in the SIM mode: m/z 212, 105 for BB corresponding to $(M)^{+\bullet} = 212$ and $(M-C_7H_7O)^+ = 105$; m/z 108, 91 for BA corresponding to $(M)^{+\bullet} = 108$ and $(M-OH)^+ = 91$.

4. Determination of benzyl benzoate loading

Ten ml of the nanoparticle suspension were centrifuged at 50,000 × g for 45 min or destabilized by addition of copper(II)sulphate (flocculation) in order to obtain a clear supernatant liquid which was then removed by aspiration. The liquid phase and the nanoparticle sediment were extracted twice with methylene chloride (50 ml). The organic phases were collected and dried over anh. sodium sulphate and then evaporated under reduced pressure. Residues were diluted with a suitable volume of methanolic solution of internal standard and then injected into the GC-MS system.

5. In vitro benzyl benzoate release studies

Five ml of nanoparticles containing 0.04 ml BB and 4 mg of PB per ml of suspension were suspended in 400 ml of phosphate buffer pH 7.4 at 37 °C. At successive time intervals, aliquots were collected and flocculated. Supernatants were extracted and mixed with a solution of internal standard in methanol. BB and PB were then measured by GC-MS (Fig.).

References

1 Donbrow, M.: Microcapsules and nanoparticles in medicine and pharmacy. CRC Press, Boca Raton/Ann Arbor/London 1991
2 Santiago, N.; Milstein, Rivera, T.; Garcia, E.; Zaidi, T.; Hong, H.; Bucher, D.: Pharm. Res. **10**, 1243 (1993)
3 Llovet, M. L.; Egea, M. A.; Valero, J.; Alsina, M. A., Garcia, M. L., Chauvet, A.: Drug Devel. Ind. Pharm. **21**, 1761 (1995)
4 Sah, H.; Smith, M. S.; Chern, R. T.: Pharm. Res. **13**, 360 (1996)
5 Radwan, A.: Drug Devel. Ind. Pharm. **21**, 2371 (1995)
6 Fesi, H.; Devissaguet, J. P.; Puisieux, F.; Thies, C.: US Patent 5, 118 (1992)
7 Berrabah, M.; Prevot, F.; Orecchionian, A. M.; Lafont, O.: J. Pharm. Biomed. Anal. **3**, 373 (1994)
8 Gautier, J. C.; Nivet, J. M.; Algranti, P.; Gilloteau, M.; Histe, M.; Lallier, M.; N'guyen-Huu, J. J.; Rusotto, R.: STP Pharma Prat. **2**, 205 (1992)

Received June 17, 1999
Accepted October 15, 1999

Mohammed Berrabah
Université Mohamed Premier
Faculté des Sciences
Département de Chimie
B.P. 524
60.000 Oujda
Maroc
Berrabah@sciences.univ-oujda.ac.ma