Notes: This part of the experiment was not included in the manuscript as it might dilute the strength of this paper.

Introduction

Salbutamol has a single asymmetric carbon and is administered as racemate although its therapeutic activity of salbutamol resides on the R-(-)-isomer with no activity attributed to the S-(+)-isomer (Brittain et al., 1979). The role of enantiomers in producing therapeutic effect and side effect needs to be addressed. The impact of formulation on enantioselective release of salbutamol is important to provide a rationale for future study of salbutamol metabolism.

However, there were reports that DMCD is able to use as a chiral selector in capillary electrophoresis (Rogan et al, 1994; Lemesle-Lamache et al., 1996). In addition, there was a claim that the release of R-isomer is preferable to use and retain S-isomer in the dosage form (ref..). Therefore, the final aim of this experiment was to investigate the chiral selectivity of cyclodextrin carrier towards salbutamol in the dissolution of dry powder formulation.

Methods

Preformed cyclodextrin-salbutamol release studies

A dissolution study was conducted using a condition described by the British Pharmacopoeia for testing salbutamol tablet

formulations with the paddle method. The apparatus was set up according to the Pharmacopoeia, the dissolution medium was 200 ml of water set at 37 °C. The paddle rotated at 50 rpm. A semipermeable membrane approximately 3 cm in diameter which was obtained from Spectrum® (Spectrapor, Intermedicell, UK) with a molecular weight cut-off from 1,000 was employed in this study. The membrane was prepared by cutting approximately 6 cm lengths of membrane which were soaked in water for about 1 h prior to use. 200 mg of the freezed dried complex was weighed into this membrane sac. The sac was then clipped at both sides to avoid leakage and placed into dissolution chamber. Samples were taken for each of the two formulations every 5 min up to 30 min. Each experiment was performed at least 6 times and the mean was calculated in each case. The samples were analysed for an amount of each salbutamol enantiomer by capillary electrophoresis under the following conditions.

Enantiomeric separation of salbutamol enantiomers were carried out employing chiral mobile phase additives in capillary electrophoresis (Bio-Rad, California, USA). The cartridges were fused silica (70 cm x 75 μm i.d.). The capillary column was first cleaned with 0.1 N NaOH for 3 min then equilibrated with 10 mM phosphate buffer pH 2.4 containing DMCD 50 mM for 3 min before a sample was loaded under pressure injection for 5 s. The detector was set at 214 nm and applied voltage was 10 kV and run time was set for 20 min. Standard concentration was 0.02-0.1 mg/ml.

Results and Discussion

As shown in Fig. 11, S-isomer was released slightly better than R-isomer (Fig. 11). From the results obtained, the enantio-selectivity of GCD to the release of salbutamol was not significantly different between S- and R-isomer. This is in an agreement with the works done by Rogan et al (1994). When the S-, R-salbutamol were released from DMCD-salbutamol complex, the S-isomer slightly released faster than R-isomer. These differences are significant at a level of 0.05. This implied that DMCD was not only increase the solubility of salbutamol

but also affected enantioselective release of salbutamol. When employing the formulation of lactose as a carrier, no stereoselectivity was achieved or evidenced (data not shown).

Brittain, R.T., Former, J.B., Marshall, R.J. (1973) Some observation of the β_2 adrenoceptor agonist properties of the isomers of salbutamol. *Br.J.Pharmacol.*, **3**, 113-120.

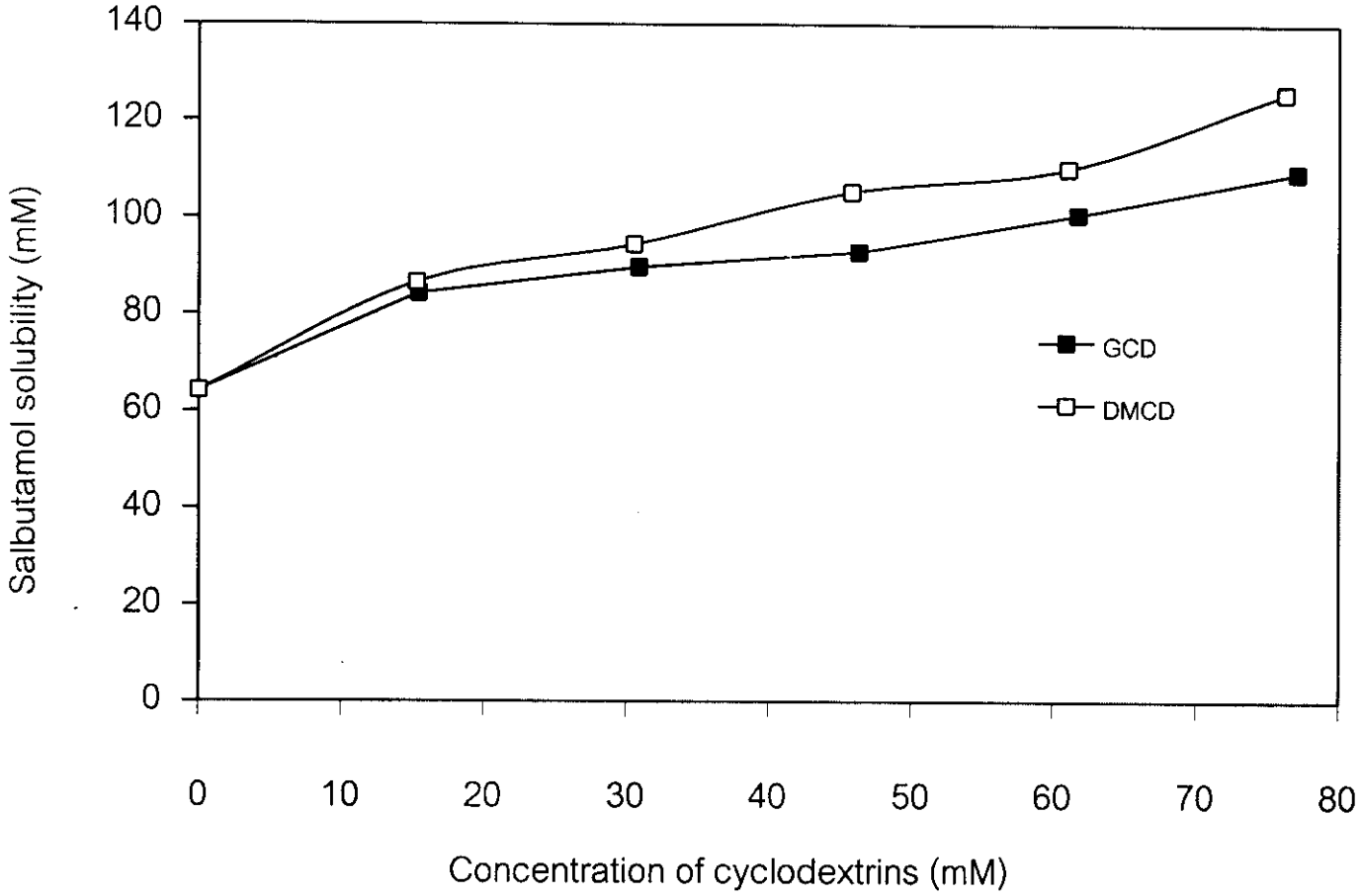

Fig. 1 Complexation curve of salbutamol and cyclodextrins [gamma cyclodextrin(GCD) and dimethyl-beta-cyclodextrin (DMCD)] (mean ± SD, n=4)

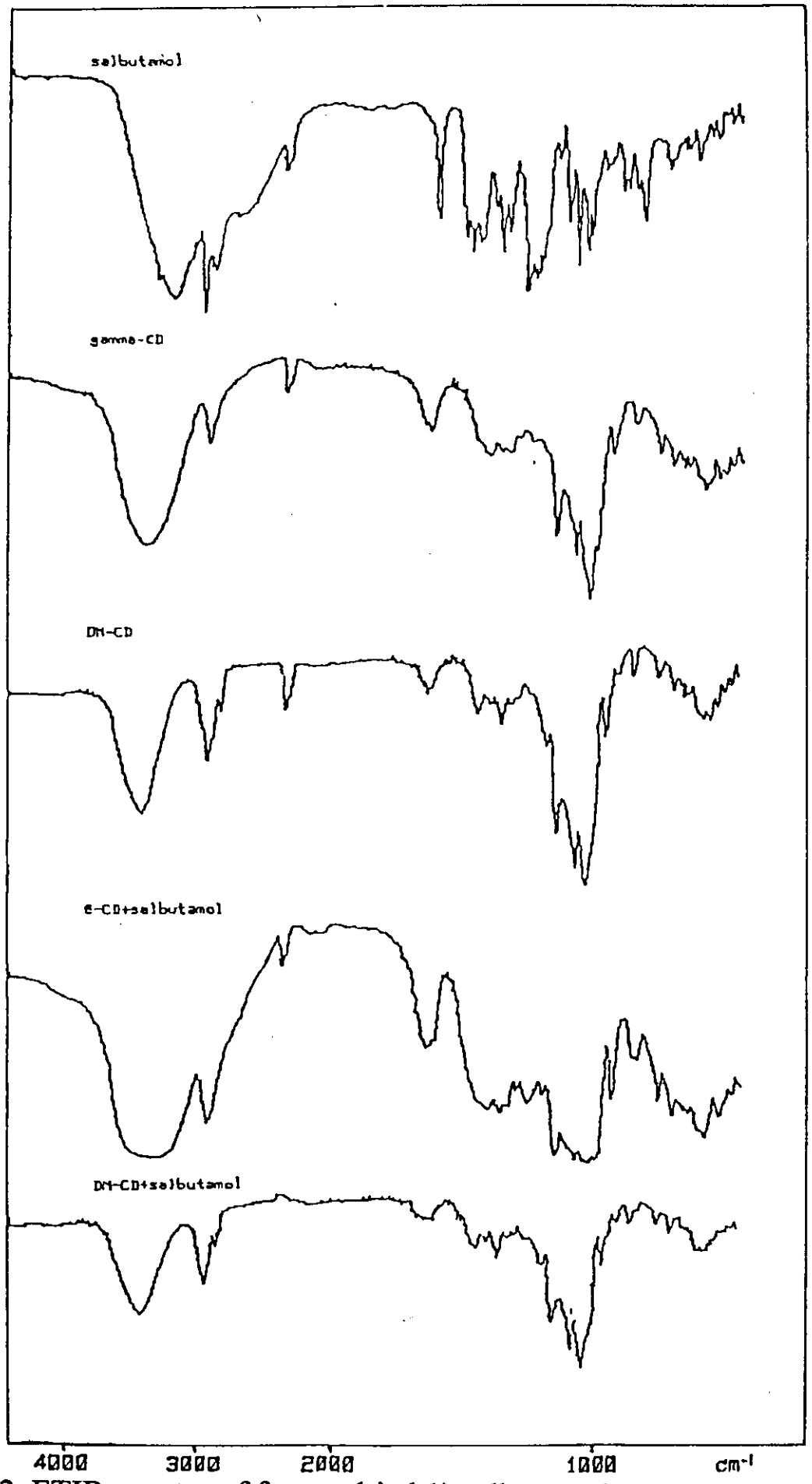

Fig 2. FTIR spectra of freeze-dried 1) salbutamol 2) gamma-CD 3) dimethyl-beta-cyclodextrin (DMCD) 4) GCD + salbutamol complex 5) DMCD + salbutamol complex

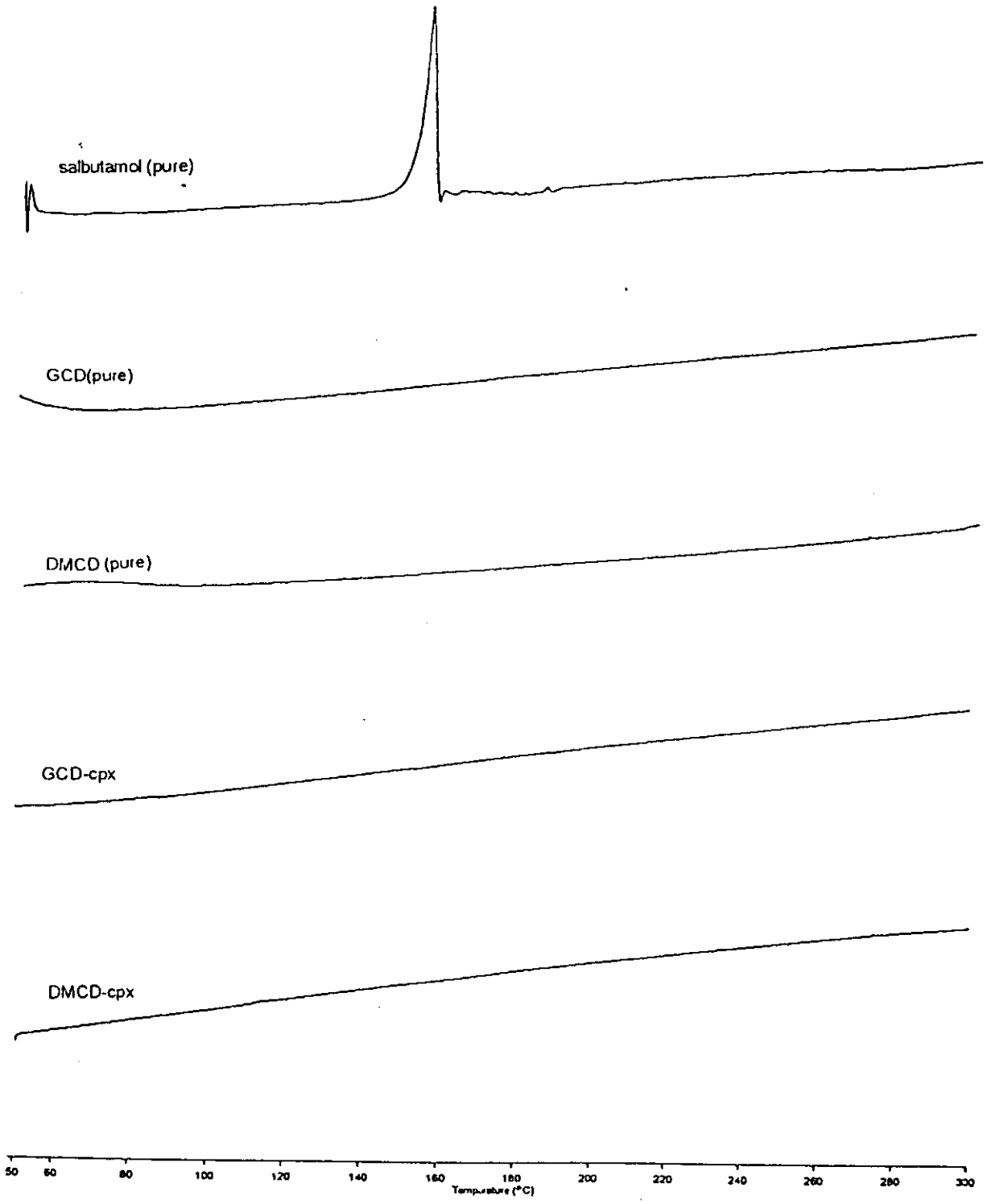

Fig 3. DSC curve of freeze-dried 1) salbutamol 2) gamma-CD 3) dimethyl-beta-cyclodextrin (DMCD) 4) GCD complex 5) DMCD complex

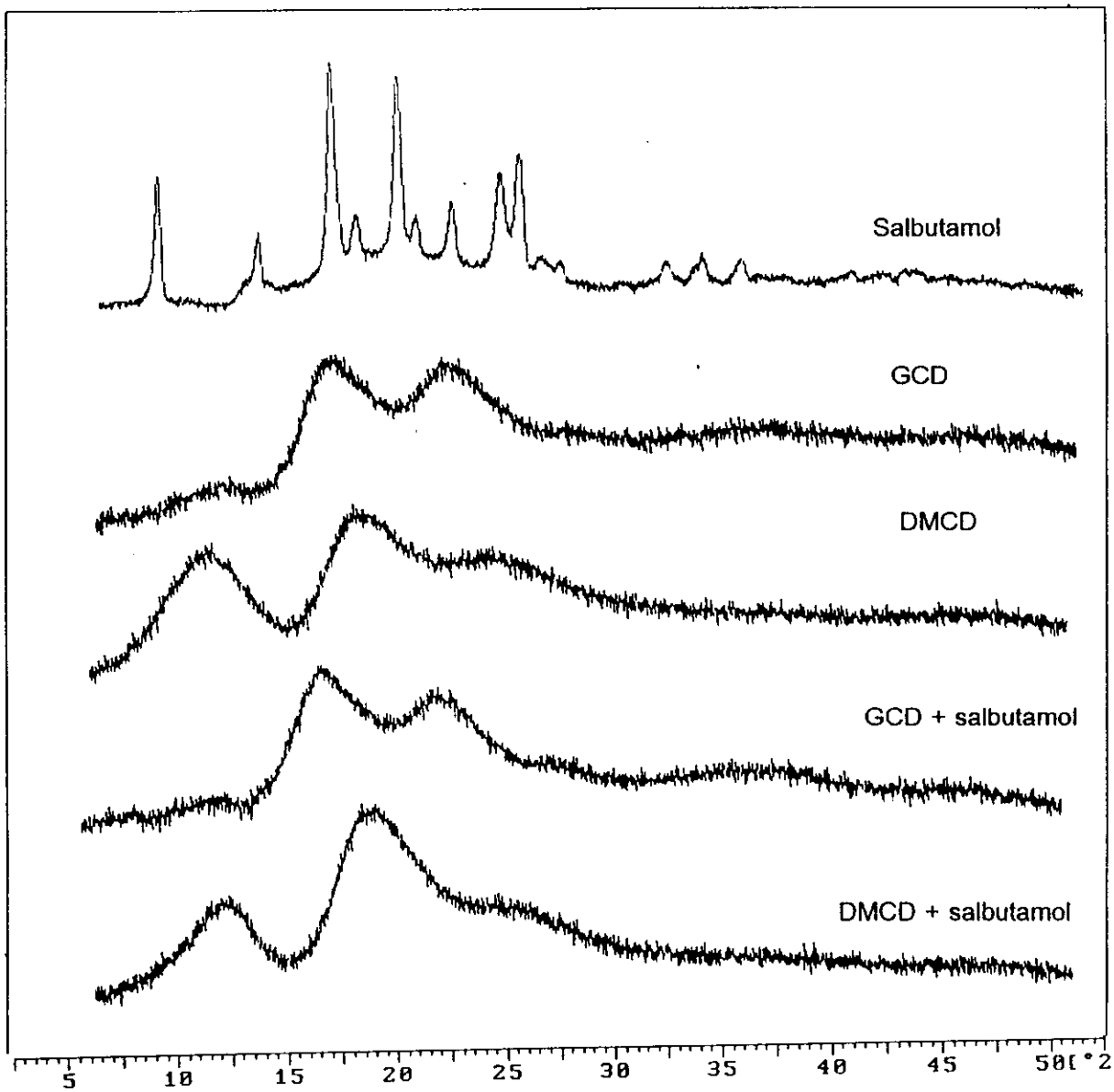

Fig 4. X-ray powder diffraction patterns of freeze-dried 1) salbutamol 2) gamma-CD 3) dimethyl-beta-cyclodextrin (DMCD) 4) GCD + salbutamol complex 5) DMCD + salbutamol complex

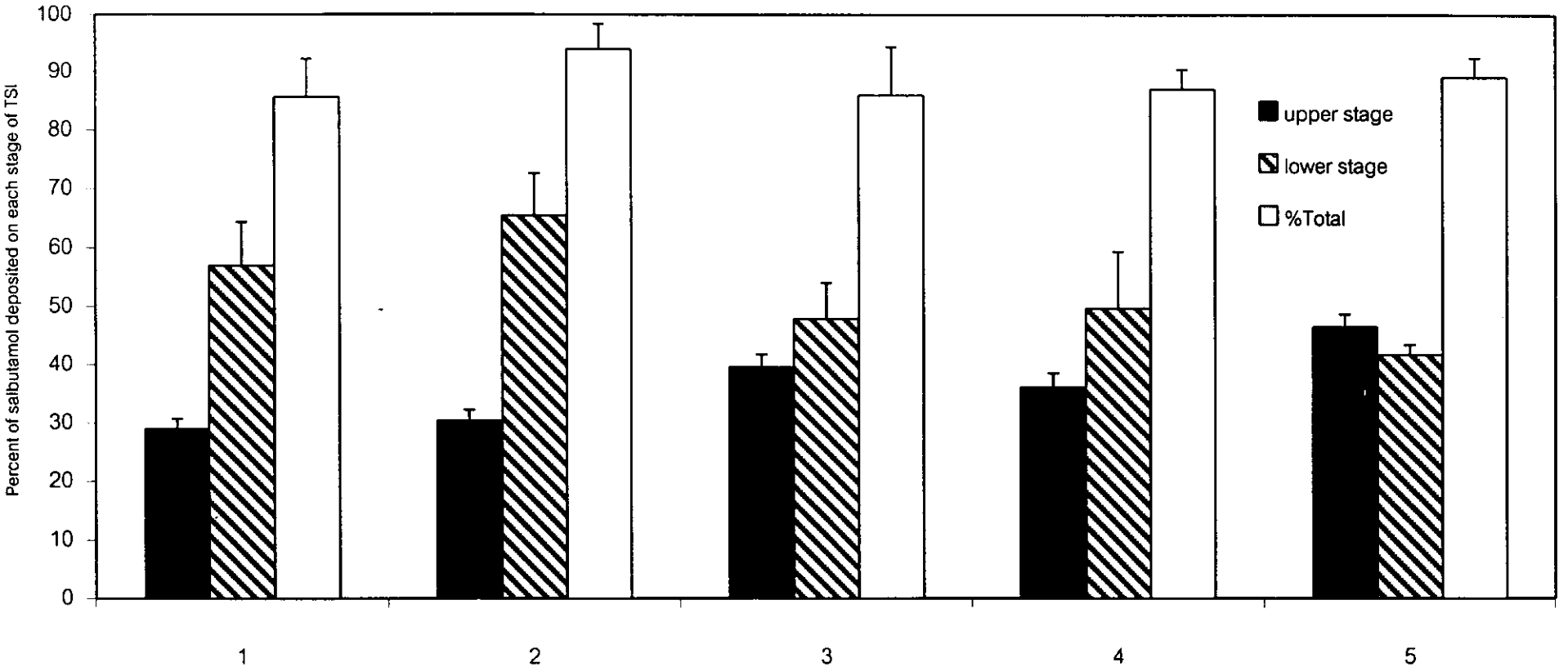

Fig. 5 Salbutamol delivered from different dry powder formulations containing [1] GCD complex mixed with lactose (1:30) [2] GCD complex mixed with lactose (1:60) [3] DMCD complex mixed with lactose (1:30) [4] DMCD complex with lactose (1:60) [5] salbutamol mixed with lactose (1:60) aerosolised at 60 l/min into twin stage impinger (TSI) (mean $\pm$ SD, n =6)

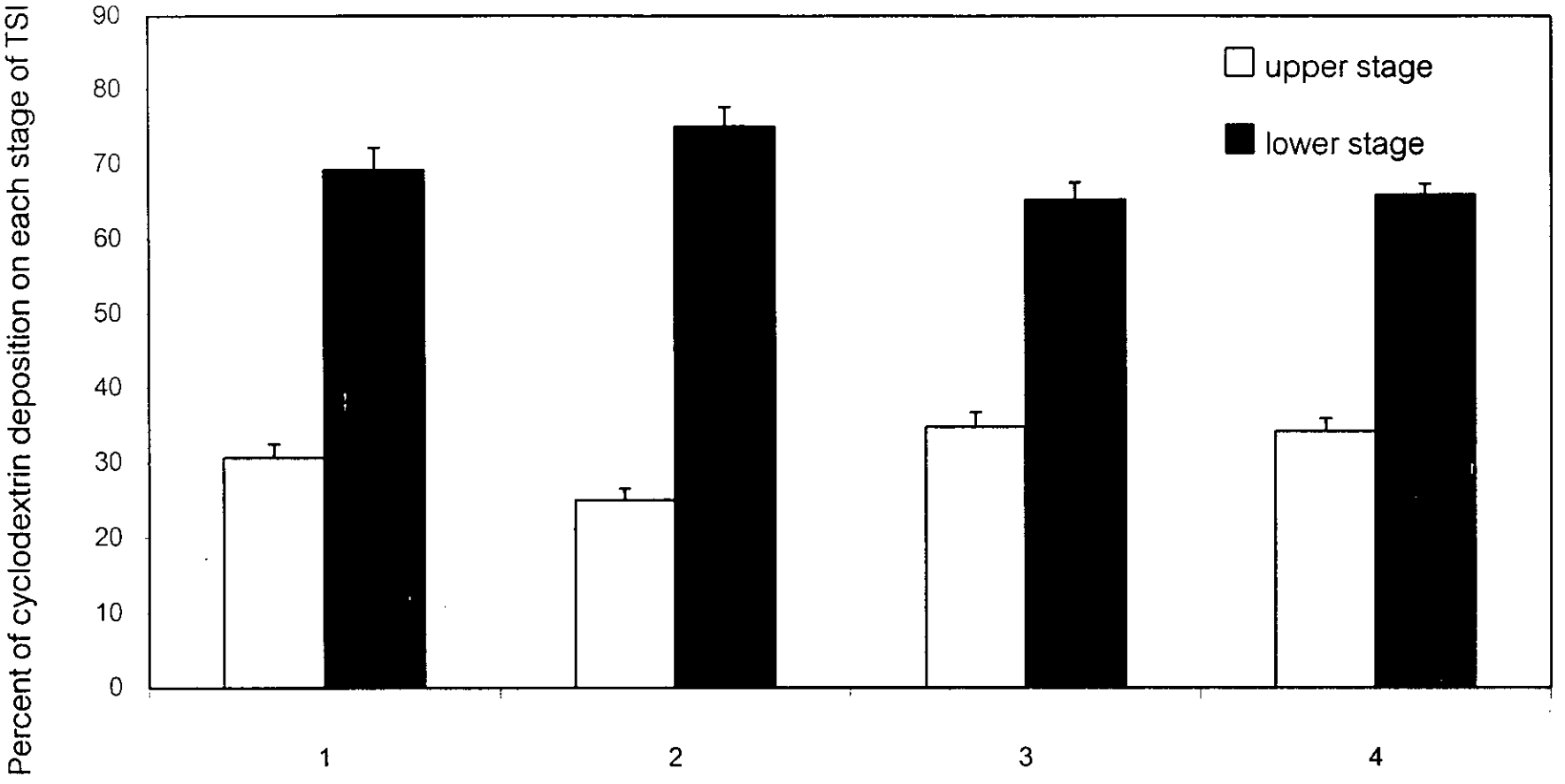

Fig. 6 Cyclodextrin deposition on each stage of TSI aerosolised from formulations containing [1] GCD complex mixed with lactose (1:30) [2] GCD complex mixed with lactose (1:60) [3] DMCD complex mixed with lactose (1:30) [4] DMCD complex mixed with lactose (1:60) (mean±SD, n =6)

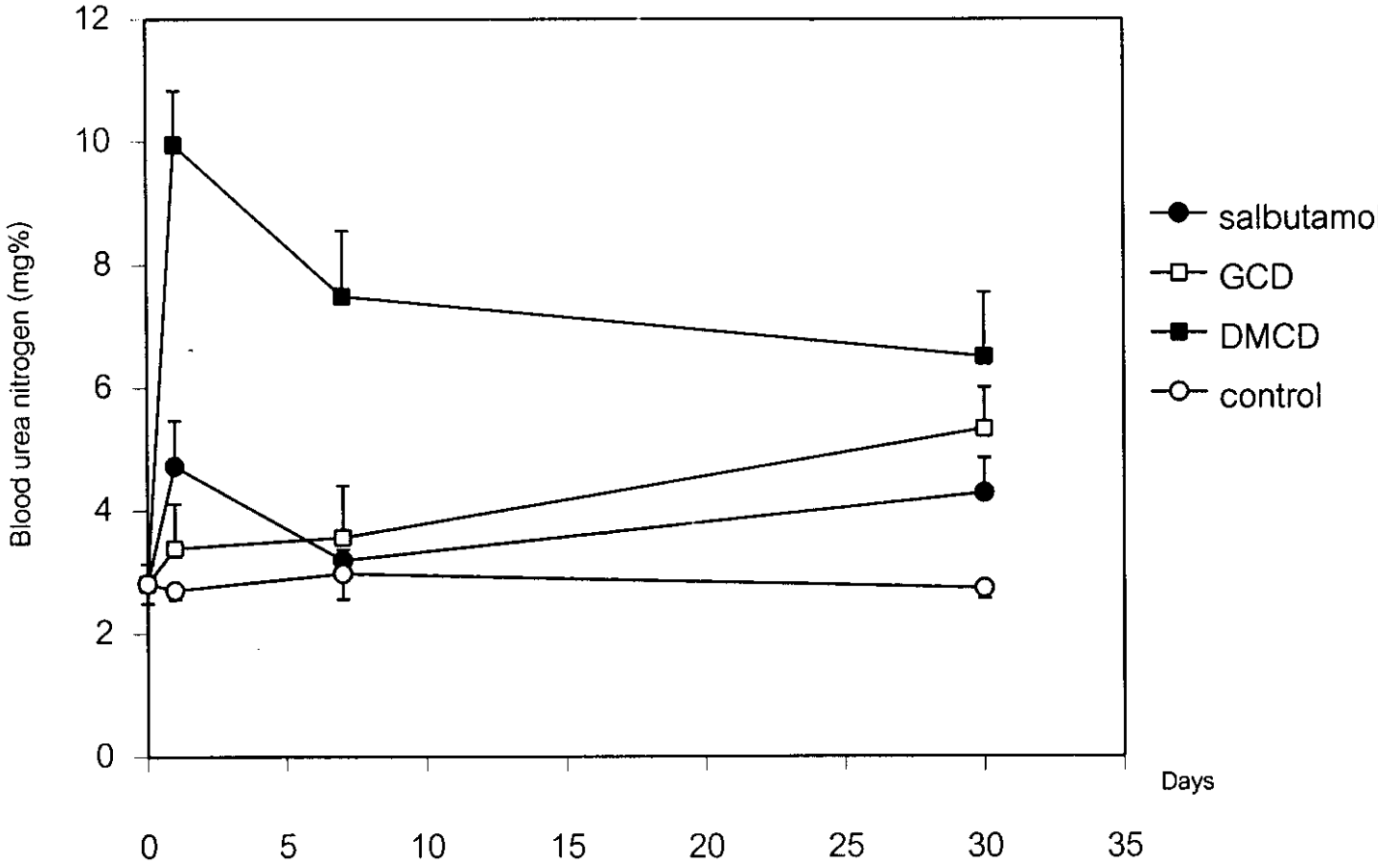

Fig. 7 Blood urea nitrogen in rat after intraperitoneal injection of salbutamol 200 ug/ml; gamma-cyclodextrins(GCD) and dimethyl-beta-cyclodextrin (DMCD) 1 mg/ml at day 0, 1, 7 and 30 (mean ± SD, n = 5)

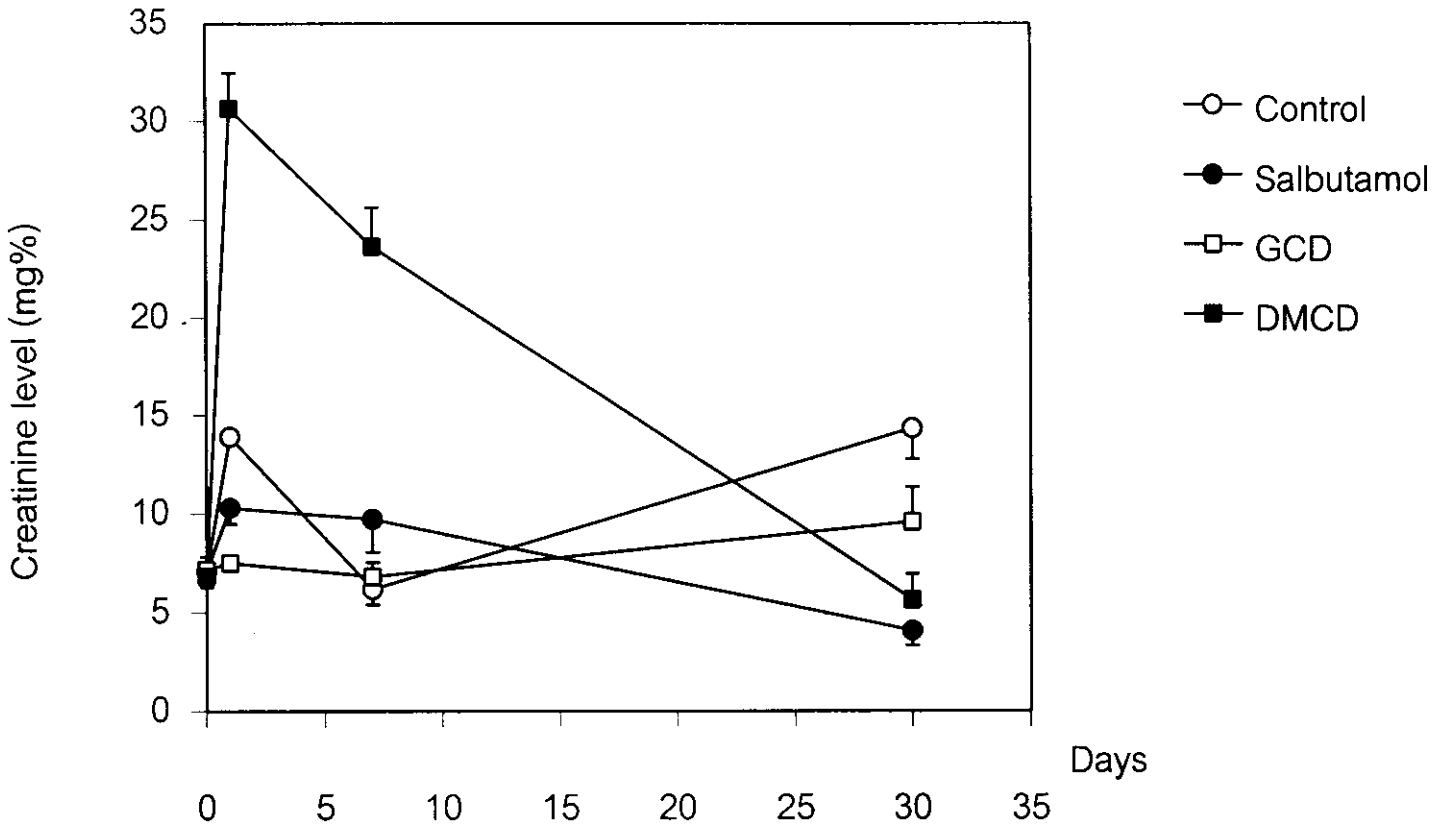

Fig. 8 Urinary creatinine in rat after intraperitoneal injection of salbutamol 200 ug/ml, gamm-cyclodextrins (GCD) and dimethyl-beta-cyclodextrin (DMCD) 1 mg/ml at day 0, 1, 7 and 30 (mean ± SD, n = 5)

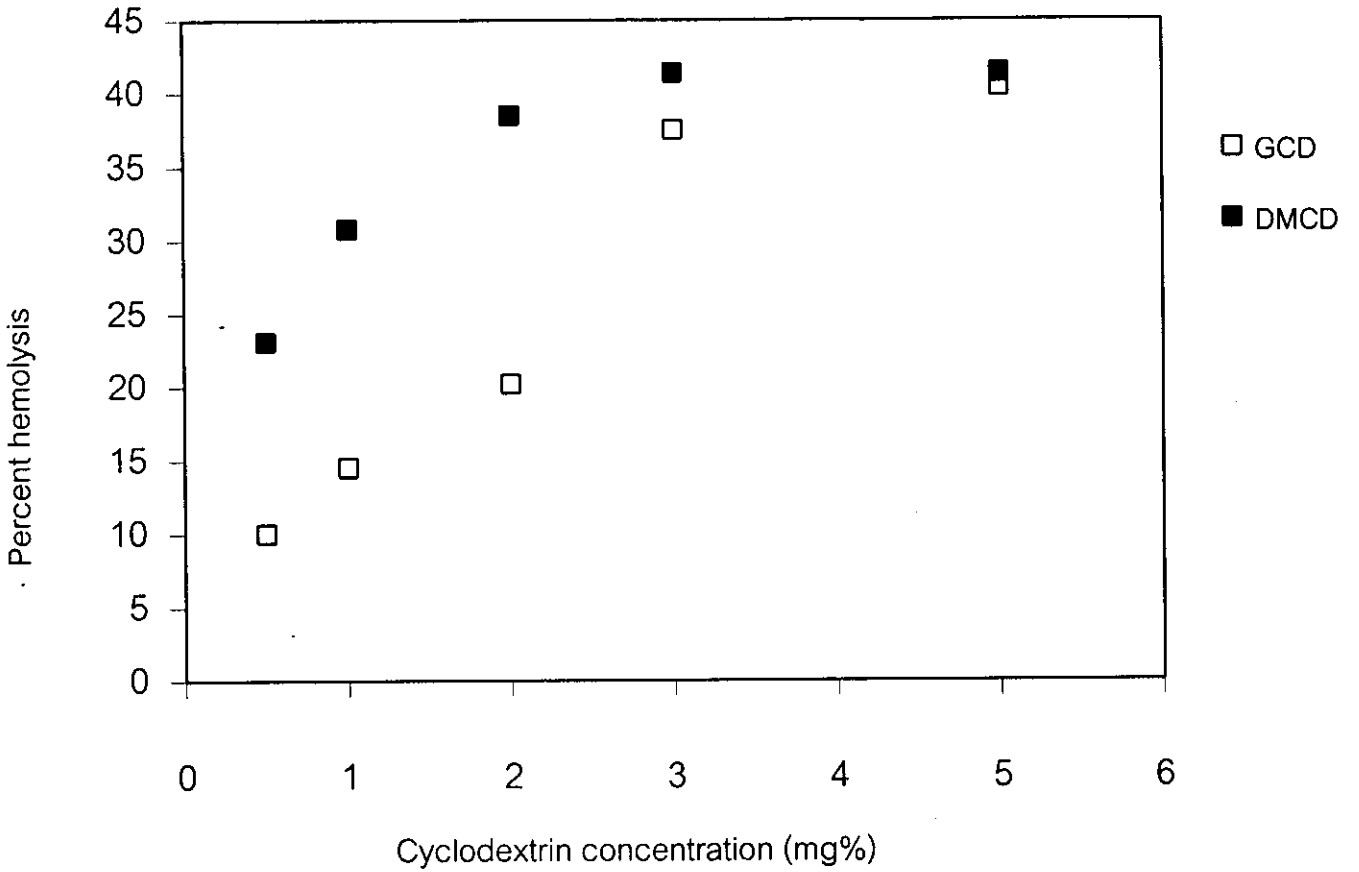

Fig. 9 Hemolysis of human red blood cells incubated in cyclodextrin solutions (GCD and DMCD) for 15 min at various concentrations

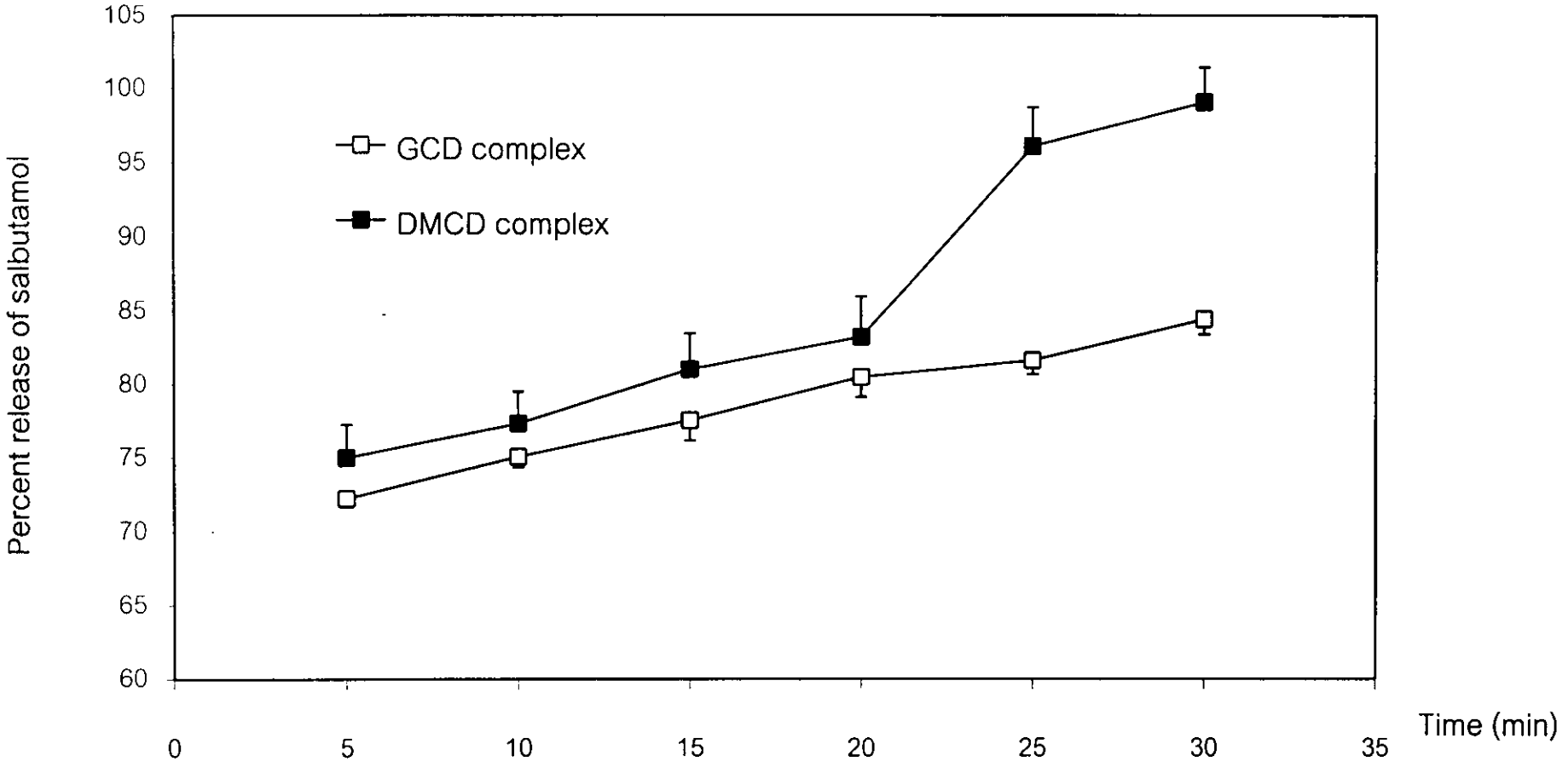

Fig. 10 The release of salbutamol from salbutamol-cyclodextrin complexes (GCD and DMCD complexes) (mean ± SD, n = 6)

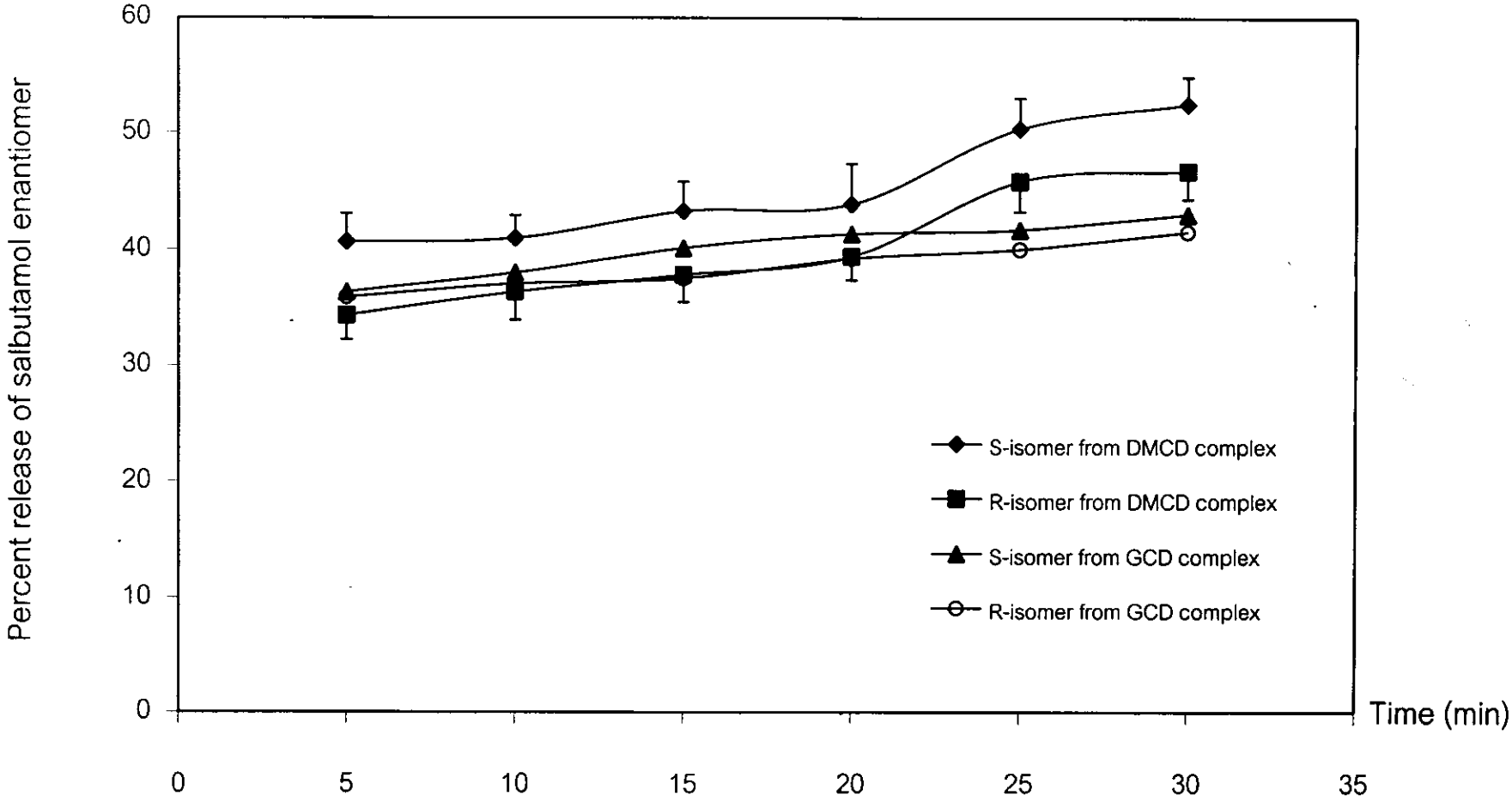

Fig 11. The release of salbutamol enantiomers from salbutamol-cyclodextrin complexes (GCD and DMCD complexes) (mean ± SD, n = 6)