Statistical analysis of data was performed by one-way ANOVA. Differences were considered to be statistically significant at $p < 0.05$.

**Results and Discussion**

The solubility of salbutamol increased in the presence of different type of cyclodextrins (DMCD, GCD) as compared to that in water (Fig. 1). The profile of salbutamol solubility ($y$) in mM was plotted as a function of cyclodextrin concentration ($x$) in mM. The slope from the linear portion of the curve was put into Higuchi equation.

$$\text{slope} = \frac{mkS_0^m}{1 + kS_0^m}$$

$m$ = molar ratio of drug to CD

$k$ = stability constant

$S_0$ = solubility of drug in the absence of CD
Complexation constant of salbutamol and DMCD was 58.55 M$^{-1}$ while the complex of GCD was 12.7 M$^{-1}$. This means that the complex formation of DMCD with salbutamol is greater than that of GCD. Since the complex powder was micronised under air jet microniser before use as an ingredient in dry powder formulation, the size was less than 5 μm. This is suitable for use as an active ingredient for lower lung delivery of dry powder inhaler.

**Cyclodextrin-salbutamol characterisation**

Intermolecular interactions such as the hydrophobic interaction, van der Waal’s force, H-bonding and other physical forces are involved in the complexation process. In this experiment, the analytical techniques such as IR, DSC and XRD were used to verify complex formation of salbutamol with the two CDs.

The typical IR spectrum of salbutamol with absorption peak at 3320 cm$^{-1}$, indicating O-H and N-H bonds is shown in Fig. 2.
IR spectra of all types of CDs (alone) revealed a broad peak characteristic of the hydroxyl group stretching vibration in the 3500-3200 cm\(^{-1}\) region. In addition, the characteristic IR spectra in the C-H stretching vibration of DMCD (methyl group band at 2875 cm\(^{-1}\)). As shown in Fig. 2, the IR spectra of the two complexes remained the peaks due to CDs but not the drug. Therefore, the formation of complexes of drug with the CDs is possible.

The thermal behavior of the solid complexes was also determined to confirm in the complexity of salbutamol and CDs. Where guest molecules are incorporated in the CD cavity or in the crystal lattice, the melting, boiling and sublimation points of the inclusions usually shift to a different temperature. The DSC curves of the freeze-dried racemic salbutamol, the freeze-dried CDs and the freeze-dried of inclusion complexes of salbutamol with the CDs are shown in Fig. 3. The DSC curve of the freeze-dried salbutamol showed an endothermic peak at 150°C, representing the melting point of salbutamol. The DSC curves
of two freeze-dried complexes showed that the endothermic peak of salbutamol disappeared within the temperature range where the CD hosts decomposed. This may be attributed to the formation of the inclusion compounds.

The XRD analysis was performed to confirm the formation of complexes of salbutamol with CDs. Fig. 4 depicts the X-ray patterns of the freeze-dried racemic salbutamol alone, the freeze-dried CDs and the freeze-dried complexes of salbutamol with CDs. The characteristic peaks of salbutamol appeared at a diffraction angle of 2θ at 8.46, 16.32, 19.69 and 25.15°. The diffraction patterns of the CDs complexes showed the disappearance of salbutamol peak. These results indicate the production of the amorphous state of salbutamol in the complexes of all CDs as thermal analysis does prove to be.

Deposition studies

After 8 disk blister containing 8 dose was loaded into the Diskhaler device (Glaxo-Wellcome, Ware, UK). The air was
drawn through the device to deliver the DPI into the TSI at a
flow rate of 60 l/min for 10 s. Drug and CD deposition on each
stage of the impinger was determined by HPLC. The total
emission from all five formulations was higher than 80%. The
drug penetrated to lower stage of formulations 1&2 was around
70% which was much higher than that of the control formulation
(the CD was absent). These results illustrate that GCD can be
used as an enhancer to increase the penetration of salbutamol
into the lower stage of the impinger. While the deposition of
salbutamol delivered from DMCD formulation was higher than
50% of the nominal dose in both formulations. In case of more
amount of lactose employed in the formulation, the drug
delivery was slightly increased as compared to that of the lower
amount of lactose formulation. Also, the in vitro deposition in
this case is much higher than ever reported FPF in the literature
(Timsina et al., 1994). Therefore, it is possible to use GCD to
enhance the delivery of salbutamol to the lower airways. This
can be promoted slightly higher drug to lower stage when higher
amount of lactose carrier was present in the formulations. The
GCD is better ingredient in delivery drug to lower airways than that of DMCD. The deposition of CD was detected both in the upper and lower stages of the impinger as shown in Fig. 6. The amount of CD in the lower stage was about 1 mg, therefore this amount was employed to further investigate in the possible toxicity of CD in vivo.

Toxicity studies of CD

When giving both types of CDs by intraperitoneal injection, the plasma urea nitrogen and urinary creatinine were monitored. The urea nitrogen level in the control group which received normal saline solution was 2-5 mg% over a period of one month (Fig. 7). When salbutamol was given, BUN was about 3-4 mg% and the urea increased after one day injection to 5% and decreased to normal level at day 7. While the rat was administered with GCD, the BUN apparently increased from day 0 to day 1 and decreased after day 7 to day 30 (Fig. 7). However, it was around 5 mg%, in case of DMCD, the BUN increased to 10 mg% at day 1 after injection which was different
from that of injecting normal saline solution, salbutamol and GCD. Then BUN decreased to 7.5% and 6.5% at day 7 and day 30, respectively. If these results were compared to other four cases, it can be concluded that the possibility of toxicity to kidney was highest with the use of DMCD. In case of GCD, toxicity was not significantly higher when compared to that of salbutamol or control. Therefore, GCD is safer in this case as compared to DMCD. However, over the concentrations used, BUN was still less than 15mg% (this value was considered to be normal). The plasma urea nitrogen was determined for DMCD and this was significantly higher than the control group and the results indicate that kidney damage was from high concentration of urea in the systemic circulation. These results suggest that the cyclodextrin employed do not cause acute renal damage under the condition studied.

The nephrotoxicity of cyclodextrin results from the crystal formed through the urinary tract. As can be seen in Fig. 8, the animal which received buffered normal saline did not show the creatinine value above that of the control. In comparison, GCD
increased creatinine to 8.15% which was not significantly different from control (P > 0.05). These values, however, were much higher for DMCD after 24 h to a level of 30 mg% and declined after one week to 24 mg% and backed to a level of control (normal) at 30 days. These results indicate that the use of GCD is quite safer than the use of DMCD, as creatinine level was considered.

For hemolysis studies, intraperitoneal administration of cyclodextrin provides contact of these agents with cellular membranes and membrane components. Although, new models for evaluating the effects of cyclodextrins on membranes have been reported (Leroy-Lechat 1994). However, *in vitro* hemolysis studies are not a true predictor for *in vivo* membrane interactions due to the dynamic association behaviors of parenterally administered cyclodextrin with endogenous compounds (Leroy-Lechat 1994). Hemolysis studies however provide rapid means for determining relative-membrane comprising ability. As can be seen in Fig. 9, the hemolysis of red blood cell in buffered isotonic solution tends to be 4.5
million cells /ml. When RBC was mixed with cyclodextrins at various concentrations in buffered isotonic, it was found that the hemolysis occurred as the concentration of cyclodextrin increased, at 0.5 mg/ml with a degree of hemolysis about 10% and at 5mg/ml with degree of hemolysis 40%. The hemolysis of RBC in case of DMCD was two times higher than that of using GCD at 0.5, 1, 2 mg%. However, at high concentration (3-5 mg%), the hemolytic rate of both GCD and DMCD was not significantly different but the DMCD was still higher than that of GCD. The hemolysis did not reach 50% hemolysis over the concentration range studied. Perhaps, this can be concluded that GCD is safer than DMCD. It is apparent that the hemolytic activity of GCD was weaker than those of DMCD. For example, hemolysis at 1 mg % of DMCD and GCD caused hemolysis of about 30% and 15%, respectively. In case of salbutamol, hemolysis of RBC was not different from that of normal saline solution. Also, light microscope indicated no changes in the shape of erythrocyte even at a concentration of 200 micrograms, the same as that in the control. These results
suggest that the cyclodextrins do not cause acute renal damage under conditions studies.
Preformed cyclodextrin-salbutamol dissolution studies

The release of salbutamol through semi-permeable membrane was tested in order to allow only drug molecule while keeping cyclodextrin molecules within the semi-permeable membrane sac. It can be seen that the total release was achieved over 80% in both types of complexes. It was relatively fast release in vitro (30 min). The formulation of salbutamol-GCD complex released slower than salbutamol-DMCD complex. This corresponded to its solubility curve in Fig. 1 that the DMCD complex dissolved faster and higher as compared to GCD complex (Fig. 10). These differences were significant at a level of 0.05.