Chapter 6
Surface Immobilization of Carbon Nanotubes by β-Cyclodextrins and Their Inclusion Ability
Chapter 6

Surface Immobilization of Carbon Nanotubes by β-Cyclodextrins and Their Inclusion Ability

6.1 Introduction

The rich chemistry of carbon allotropes and methods for their commercial mass-production has attracted and inspired scientists of all over the world to evaluate them for a wide range of applications\(^1\text{-}^3\). Among the commercially available carbon allotropes, carbon nanotubes (CNTs) show exceptional applications in materials science, nanotechnologies and medicinal chemistry owing to their unique electrical, mechanical, ultra-light weight and thermal properties\(^4\text{-}^6\). The excellent electron affinity and charge transfer capability make CNTs attractive for a number of various applications such as adsorbent, catalyst, bio-sensing electrode, solar cell, photo sensitizer, super conducting material, gas sensor, transistor, hydrogen storage and so on\(^7\text{-}^{12}\). However, the vital biomedical applications of CNTs are still under developed due to major barriers i.e. limited reactivity and water-insolubility owing to their strong π-π stacking interactions and high aspect ratio\(^13\text{-}^{16}\). Thus, the extensive research is being undertaken to develop new dispersion techniques, which allows using the properties of CNTs in water and other polar solvents\(^17\text{-}^{20}\). Several covalent and non-covalent strategies have been attempted for the dispersion of CNTs in water and other organic solvents\(^21\). In covalent approaches, carboxylic acid, halogenated and azide functionalized CNTs have been effectively attached to the solubilizing agents\(^22\). In non-covalent approaches, CNTs were effectively wrapped by aromatic molecules, polymers and surfactants\(^23\text{-}^{25}\). However, the demerit of using non-covalent interactions to make CNTs hybrids is that in a competitive reaction medium the adsorbed molecule could be easily displaced from the CNTs surface\(^26\). In general, for pursuing the practical applications of CNTs, the dispersion of CNTs in different solvents continues to be a challenge.

Recently, the considerable interest has been devoted for the dispersion of CNTs\(^27\text{-}^{28}\). Despite the several methods described for the preparation of water-dispersible CNTs in literature; efforts are continuing to find simpler, biocompatible and more inexpensive routes for functionalization of CNTs in order to elevate their
dispersibility. The surface modification of CNTs through covalent-noncovalent functionalization with supramolecular molecules is crucial for developing high performance materials based on CNTs and supramolecular materials\(^{29}\). The controlled covalent-noncovalent functionalization of CNTs with cyclodextrins improves their dispersibility with the simultaneous retention of their unique characteristic properties\(^{30,31}\). Cyclodextrins has been effectively used for the soft cutting of CNTs\(^{32}\).

Cyclodextrins are well known to serve as good host molecules for the aqueous solubilization of carbon allotropes\(^{33,34}\). The covalent attachment of cyclodextrin molecules on the surface of CNTs is an effective option of bringing the guest molecule closer to the CNTs surface and thus enhancing the interaction between the guest and CNTs\(^{35}\). Recently, it was realized that combination of cyclodextrins and carbon materials by covalent functionalization generates a new class of materials with more advanced properties and applications than the pristine precursors and non-covalent interaction\(^{36}\). The covalent attachment of molecules to CNTs broadens the range of potential applications of CNTs systems and allows one to tailor the properties of modified CNTs\(^{7}\). The β-cyclodextrin is most common, cheapest and widely used among the commercial available cyclodextrins. Selective replacement of hydroxyl groups with other functional groups have been shown to remarkably improve the reactivity and solubility of the native β-cyclodextrin. However, presence of similar hydroxyl groups makes selective modification of β-cyclodextrin extremely difficult, although regioselective modification of β-cyclodextrin was achieved by using geometry of reagents, which can result in a selectively difunctionalized β-cyclodextrin to improve water-solubility, moderate inclusion ability and reactive functionality\(^{51}\).

Investigations of the photophysical properties of photosensitive molecules on CNTs are of interest as they provide the scientific basis for hybrid materials with potential applications as optical sensors\(^{38,39}\) and light harvesting devices\(^{40}\). Cho et al developed a CNT-chromophore based pH sensor in which a fluorescent dye (pyrene) was attached to the surface of CNT by a pH sensitive polymeric linker that coils up at low pH and causes quenching of the dyes\(^{38}\). CNTs have been used as substrates in immunoassays. CNT-electrodes were decorated with antibodies that can bind antigens, allowing an electrochemiluminescence-active second antibody to bind,
thereby enabling detection of the antigen. CNT-polymer hybrids and CNT-phthalocyanine hybrids have been developed as light harvesting devices.

This chapter describes the surface immobilization of CNTs with β-cyclodextrins along the wall of CNTs using covalent amide linkages. For this purpose, both β-cyclodextrin and CNTs were functionalized. Thus, β-cyclodextrin was modified with reactive amino functional groups and CNTs were functionalized to generate carboxylic groups on the surface. The amine functionality on the primary side of the β-cyclodextrin was effectively used, as it can react with carboxylic group of CNTs via amide linkage. The presence of β-cyclodextrin on the surface of CNTs can provide not only excellent water-dispersibility to the CNTs but also site for inclusion of guest molecules or ions for interaction with the nanotube surface.
6.2 Experimental

6.2.1 Materials

Selectively diamino-functionalized β-cyclodextrin was synthesized in Chapter 2 used as reagent. MWCNTs were purchased from Wako Pure Chemical Industries, Ltd. and purified by refluxing in concentrated (69 %) HNO$_3$ for 2 days, followed by washing with concentrated hydrochloric acid and then water on a Millipore VC membrane (pore size = 0.1 μm). 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC) and phosphotungstic acid was purchased from Sigma-Aldrich Chemical Co. Rhodamine 6G was a product of Acros Chemical Co. All other chemicals were commercial products of reagent grade and used without further purification. Water was purified by distillation and deionized using a Millipore Milli-Q Laboratory purification system (resistance greater than 18.2 MΩ) and used throughout the experiments.

6.2.2 Measurements

FTIR spectroscopic measurements were recorded on a Nicolet-6700 spectrometer at 10$^{-4}$ resolution and 32 scans using a KBr pellet method. Thermogravimetric analyses (TGA) were done on a TGA Q500 instrument with a heating rate of 10°C/min in the mixed atmosphere of nitrogen and oxygen in the ratio 60/40. The thermobalance was calibrated with an alumel alloy and nickel for temperature settings and with a 100 mg standard for weight accuracy. Sample (5.5 ± 0.5 mg) was placed on a tarred aluminum balance pan and transferred to the furnace at room temperature, where the exact sample weight was determined. The measurements of energy-dispersion X-ray spectrometry (EDS) of powders on the carbon tape after gold-coating were performed on a JEOL JSM-6500F at an operation voltage of 20 kV. Transmission Electron Microscopic (TEM) images were obtained on a Hitachi H-700 equipped with a charge-coupled device camera, operating at an accelerating voltage of 100 kV. The specimens for TEM were prepared by directly dropping the dispersion of corresponding products onto the carbon-coated copper grids and dried under bulb. Fluorescence spectra were recorded on a HITACHI F-3010 fluorometer with a 10 mm path cell.
6.2.3 Carboxylic acid Functionalization of MWCNTs

Carboxylic acid-functionalized MWCNTs (CNTs-COOH’s) were obtained by refluxing 20 mg of purified MWCNTs in 20 cm$^3$ concentrated HNO$_3$/H$_2$SO$_4$ (3:1, v/v) for 24h after 2h sonication. After centrifugation, washing several times with water and drying overnight in vacuum oven at 60° C, the solid obtained was subjected to FTIR analysis to check the effective surface functionalization of MWCNTs with carboxylic groups.

6.2.4 Surface Immobilization of MWCNTs with β-Cyclodextrins

The carboxylic acid functionalized MWCNTs were immersed into 30 cm$^3$ of a freshly prepared 10 mM EDC solution to convert the carboxylic groups of MWCNTs into active ester (2 in Scheme 6.2). After activation for 30 min, 100 mg of diamino β-cyclodextrin was added and the reaction mixture was allowed to stir for 24h at room temperature. The excess of diamino β-cyclodextrin and byproducts obtained during the progress of reaction were removed by washing with water and dialyzing with a polymer membrane (MWCO 2K) for 24h. The product, denoted as β-cyclodextrin-modified MWCNTs, was obtained by centrifugation and drying at 60° C for 24h. The reaction and mechanism is shown in Scheme 6.1 and Scheme 6.2, respectively.

![Scheme 6.1 Synthetic reaction scheme of chemical immobilization of β-cyclodextrins on MWCNTs.](image-url)
Scheme 6.2 Mechanism for the reaction of diamino $\beta$-cyclodextrin with oxidized MWCNT by an activated ester intermediate.

6.2.5 Sample Preparation for Fluorescence Measurement

A $5.0 \times 10^{-5} \text{ M}$ rhodamine 6G solution and a $25 \mu\text{g/mL} \ \beta$-cyclodextrin-modified MWCNTs solution were prepared in water. The rhodamine 6G solution (1 mL) was added to the different volumes (1-9 mL) of a $\beta$-cyclodextrin-modified MWCNTs solution and then adjusted to a total volume of 10 mL by adding water. The mixtures were stirred and then allowed to equilibrate for 8h before fluorescence measurements. Thus, the final concentration of rhodamine 6G was constant ($5.0 \times 10^{-5} \text{ M}$) and the concentration of $\beta$-cyclodextrin-modified MWCNTs was varied between 0-22.5 $\mu\text{g/mL}$.
6.3 Results and Discussion

6.3.1 Characterization of β-Cyclodextrins Immobilized on MWCNTs

6.3.1.1 Fourier Transform Infrared Spectroscopy (FTIR)

β-cyclodextrin-modified MWCNTs was synthesized by covalent attachment of oxidized MWCNTs with diamino β-cyclodextrin (Scheme 6.1) through amide linkages using water-soluble condensation agent EDC. Figure 6.1a shows a FTIR absorption spectrum of oxidized MWCNTs. The spectrum exhibited bands at 1720 and 1560 cm\(^{-1}\) due to -C=O stretching and -COO\(^{-}\) antisymmetric stretching vibration modes, respectively, indicating the presence of carboxylic acid and carboxylate groups on the surface of MWCNTs after treatment with acid mixture\(^{43,44}\). The bands at 3440 and 1640 cm\(^{-1}\) come from traces of water. The covalent amide linkages between MWCNTs and modified β-cyclodextrin were confirmed from the FTIR spectra shown in Figure 6.1b and 6.1c. In the spectrum of diamino β-cyclodextrin (Figure 6.1b), the absorption bands were found at 3368, 2920, 1660, 1155, and 1029 cm\(^{-1}\), which could be assigned to the typical hydrogen bonded –OH stretching, -C-H stretching, -OH bending band, -C-O stretching and –C-O-C stretching absorption bands of β-cyclodextrin. Incidentally, the FTIR absorption spectrum of β-cyclodextrin-modified MWCNTs exhibited similar absorption bands to β-cyclodextrin, indicating the binding of β-cyclodextrin on MWCNTs and the retaining of the pristine structure of β-cyclodextrin after the modification with MWCNTs. Besides, there was disappearance of absorption bands at 1720 and 1560 cm\(^{-1}\) of oxidized MWCNTs was observed. Moreover, the typical peak of amide band at 1674 cm\(^{-1}\) was hindered over the typical broad peak of β-CD. These results clearly suggest that carboxylic groups of MWCNTs were completely exhausted by chemical reaction with diamino β-cyclodextrin, confirming the successful attachment of diamino β-cyclodextrin onto the surface of CNTs through covalent amide linkages.
Figure 6.1 FTIR spectra of (a) oxidized MWCNTs, (b) diaminobeta-cyclodextrin and (c) beta-cyclodextrin-modified MWCNTs

6.3.1.2 Dispersibility

One of the very convincing and visible evidence of surface modification of MWCNTs with beta-cyclodextrins was the water-dispersibility of these nanotubes. A comparison of the dispersibility is shown in Figure 6.2. Same quantities of all the three samples of (a) pristine MWCNTs, (b) oxidized MWCNTs and (c) beta-cyclodextrin-modified MWCNTs were suspended in equal volume of water and stirred for half an hour and left as it is. Within few minutes the pristine MWCNTs settled down to the bottom of the petri dish, whereas the oxidized MWCNTs did not precipitate at short time like one hour but settled down after 5h. In the case of beta-cyclodextrin-modified MWCNTs, the MWCNTs were stable with no visible precipitation in the aqueous system and product was not settled down even after several months.
Figure 6.2 Dispersibility of (a) pristine MWCNTs, (b) oxidized MWCNTs and (c) \( \beta \)-cyclodextrin-modified MWCNTs.

6.3.1.3 Thermogravimetric Analysis (TGA)

To further confirm the surface modification of MWCNTs, the obtained product was analyzed by TGA and compared to the starting materials i.e. diamino \( \beta \)-cyclodextrin and oxidized MWCNTs. Figure 6.3 shows the TGA curves of oxidized MWCNTs, diamino \( \beta \)-cyclodextrin and \( \beta \)-cyclodextrin-modified MWCNTs. As shown in Figure 6.3a, the oxidized MWCNTs showed weight loss at the temperature range of 500-700°C. On the other hand, diamino \( \beta \)-cyclodextrin (Figure 6.3b) displayed weight loss in three steps including the weight loss of contaminants (and/or water) and was completely decomposed up to 500°C. On a TGA curve of the \( \beta \)-cyclodextrin-modified MWCNTs (Figure 6.3c), the weight loss happened through some steps up to 630°C. Then weight content of \( \beta \)-cyclodextrin in \( \beta \)-cyclodextrin-modified MWCNTs was determined by weight comparison in the TGA curves at 500°C, and evaluated to be about 69 wt%. Therefore, the weight content of covalently bonded diamino \( \beta \)-cyclodextrin was estimated to be \( \sim \) 69 wt%.
Figure 6.3 TGA curves of (a) oxidized MWCNTs, (b) diamino $\beta$-cyclodextrin and (c) $\beta$-cyclodextrin-modified MWCNTs.

6.3.1.4 Energy Dispersive Spectroscopy (EDS)

EDS analysis was also carried out on the pristine MWCNTs, oxidized MWCNTs and $\beta$-cyclodextrin-modified MWCNTs and the results are given in Figure 6.4. The EDS results are quite convincing in the sense that the carbon content is reducing from the pristine MWCNTs to the oxidized MWCNTs and the $\beta$-cyclodextrin-modified MWCNTs with a corresponding increase in the elemental content of oxygen owing to effective oxidation of MWCTNs and surface immobilization of diamino $\beta$-cyclodextrins on MWCTNs.
Figure 6.4 FE-SEM combined EDS of (a) pristine MWCNTs, (b) oxidized MWCNTs and (c) β-cyclodextrin-modified MWCNTs.

6.3.1.5 Transmission Electron Micrograph (TEM)

The visible and direct confirmation of successful surface immobilization of diamino β-cyclodextrins on the surface of MWCNTs comes from the Transmission Electron Microscopic images. Figure 6.5 shows the TEM images of pristine MWCNTs, oxidized MWCNTs, β-cyclodextrin modified MWCNTs without staining and β-cyclodextrin modified MWCNTs staining with phosphotungstic acid. From the TEM images of pristine MWCNTs (Figure 6.5a) and oxidized MWCNTs (Figure 6.5b), we can observe the surface cleaning after treatment with acid mixture at reflux temperature; acid treatment process can remove extra metal catalyst, amorphous carbon and carbon nanoparticles which were formed during the course of production of MWCNTs. In the case of β-cyclodextrin-modified MWCNTs without staining (Figure 6.5c) it was difficult to see the surface immobilization of MWCNTs with β-
cyclodextrins although some haziness can be observed on the surface of MWCNTs ascribed the surface functionalization of MWCNTs with diamino \( \beta \)-cyclodextrin. In the case of \( \beta \)-cyclodextrin-modified MWCNTs staining with phosphotungstic acid (Figure 6.5d), results in black stains (high contrast spots) on the surface of MWCNTs. This is due to the adsorption of phosphotungstic acid on the amide linkages. From the TEM images it can be seen that the acid treatment and surface immobilization of MWCNTs with \( \beta \)-cyclodextrins does not damage the surface of MWCNTs.

Figure 6.5 TEM images of (a) pristine MWCNTs, (b) oxidized MWCNTs, (c) \( \beta \)-cyclodextrin-modified MWCNTs without staining and (d) \( \beta \)-cyclodextrin-modified MWCNTs staining with phosphotungstic acid.
6.3.2 Inclusion Ability of β-Cyclodextrins Immobilized on MWCNTs

6.3.2.1 Fluorescence Spectroscopy

One of the efficient methods to confirm the functionality (especially inclusion ability) of β-cyclodextrin on the CNTs surface is to see if any organic molecule can be included into the β-cyclodextrin cavity and probed with a suitable technique. Thus, the fluorescence emission of rhodamine 6G excited at 480 nm in aqueous solutions containing various concentrations of diamino β-cyclodextrin, oxidized MWCNTs and β-cyclodextrin-modified MWCNTs, respectively, was recorded. The results of these measurements are shown in Figure 6.6. The maximum emission intensity was observed at 554 nm for all systems.

![Fluorescence spectra](image)

**Figure 6.6** Fluorescence spectra (excited at 480 nm) of rhodamine 6G (5 x 10^{-6} M) in aqueous solutions of (A) diamino β-cyclodextrin, (B) pristine MWCNTs, (C) oxidized MWCNTs and (D) β-cyclodextrin-modified MWCNTs at different concentrations: (a) 0, (b) 2.5, (c) 5.0, (d) 7.5, (e) 10.0, (f) 12.5, (g) 15.0, (h) 17.5, (i) 20.0 and (j) 22.5 μg/mL.
In the presence of pristine diamino β-cyclodextrin, the fluorescence intensity of rhodamine 6G increased with increasing concentration of diamino β-cyclodextrin as expected (Figure 6.6A). This phenomenon was observed due to the formation of inclusion complex which due to the rotation of the rhodamine 6G molecule is hindered, and the relaxation of the solvent molecules is considerably decreased. Both of these effects can result in a decrease in the vibrational deactivation. The non-polar cavity of β-CD protects the quenching of the rhodamine 6G emission by water molecule. The CDs cavity behaves similarly to the organic solvent which gives non-polar surrounding for the included molecule. This altered microenvironment can provide favorable polarity for enhanced quantum efficiencies and hence the intensities of fluorescence increase. Though rhodamine 6G is not a small molecule to include into a single β-cyclodextrin unit, it can be included into two β-cyclodextrin units (Scheme 6.3a), thus forming a 2:1 complex with β-cyclodextrin and leading to the increase in the fluorescence intensity. Meanwhile, the fluorescence intensity was reduced in the presence of pristine MWCNTs, oxidized MWCNTs and β-cyclodextrin-modified MWCNTs (Figure 6.6B, 6.6C, 6.6D). The mechanism of fluorescence hypochromic effect of rhodamine 6G by the pristine nanotubes (Scheme 6.3b) can be explained by the wrapping of the rhodamine 6G on six-ringed structure of the nanotubes surface. This is expected for pristine CNTs in the aqueous solution, since the strong π-π stacking interaction between the rhodamine 6G and the surface of the CNTs results in the fluorescence quenching. The similar fluorescence reduction of rhodamine 6G on oxidized MWCNTs and β-cyclodextrin-modified MWCNTs is the indication to the fact that though covalent functionalization of the CNTs has taken place and the modification of the π structure on the surface, the CNTs still retains the essential property of the nanotubes, as the inner tubes are still intact.

Especially, the reduction of the fluorescence intensity of rhodamine 6G in the presence of the β-cyclodextrin-modified CNT is a surprising result, because apparently the rhodamine 6G molecule is not included into the cavities of two β-cyclodextrins or in other words the 2:1 inclusion complex is not formed in this case (Scheme 6.3c). This could be derived from the reason that the β-cyclodextrins are now tethered on the nanotubes surface and are not as mobile or ‘free’ as in the case of the pristine diamino β-cyclodextrin. Therefore, rhodamine 6G molecule is partially
included into non-polar cavity of β-cyclodextrin and also adsorbed on the surface of MWCNTs.

Scheme 6.3 Schematic illustrations of the formation of β-cyclodextrin-dye inclusion complex and the adsorption of dyes on MWCNTs.

Additional insights were obtained from the analysis of the fluorescence intensity depending on concentration of rhodamine 6G. It can be seen from Figure 6.7 that the emission intensity at 554 nm of rhodamine 6G decreases for all the three (a) pristine MWCNTs, (c) oxidized MWCNTs and (d) β-cyclodextrin-modified MWCNTs. However, the profile of the decrease varies for all three. While the intensity decreases linearly with concentration of pristine MWCNTs, at the concentration beyond 2.5 μg/mL for the cyclodextrin-modified MWCNTs, it shows a linear decrease with a similar slope to that for pristine MWCNTs. However, the initial decrease with a steeper slope exists at the concentration below 2.5 μg/mL for the β-cyclodextrin-modified MWCNTs. This indicates that the mechanism of the initial fluorescence hypochromism results from a different origin. The possible mechanism of the fluorescence hypochromism at the initial concentration is strong π-π stacking interaction of rhodamine 6G with well dispersed β-cyclodextrin-modified MWCNTs.
The schematic illustration of the complexes between rhodamine 6G and β-cyclodextrin-modified MWCNTs is given in Scheme 6.3.

![Graph](image)

**Figure 6.7** Change in fluorescence intensity at 554 nm of rhodamine 6G (5 x 10^{-6} M) as a function of concentration of (a) pristine MWCNTs, (b) diamino β-cyclodextrin, (c) oxidized MWCNTs and (d) β-cyclodextrin-modified MWCNTs in water.

The fluorescence hypochromism of oxidized MWCNTs also shows two steps but the slopes are different from those of β-cyclodextrin-modified MWCNTs. Since the oxidized MWCNTs has many carboxylic acids on MWCNTs surface, cationic rhodamine 6G can electrostatically bind with carboxylic acids on MWCNTs in the initial stage and sequentially may give rise to the π-π stacking interaction with the surface of the CNT, although the latter interaction should be affected by the coexisting electrostatic interaction and/or carboxylic acid.
The hypochromism efficiency can be estimated from the Stern-Volmer constants which were evaluated from the Stern-Volmer equation given below and plots given in Figure 6.8.

**Stern-Volmer equation**

\[
\frac{I_0}{I} = 1 + K_{sv}[Q]
\]

(6.1)

Where, \(I_0\) and \(I\) are the fluorescence intensity of Rhodamine 6G in absence and presence of quencher \([Q]\), \(K_{sv}\) and \([Q]\) are Stern-Volmer quenching constant and concentration of quencher, respectively. These constants are listed in Table 6.1. The constant for the \(\beta\)-cyclodextrin-modified MWCNTs was 1.6 and 23 times higher than that for the pristine MWCNTs and the oxidized MWCNTs, respectively.

![Graph](image)

**Figure 6.8** Stern-Volmer plots for (a) pristine MWCNTs, (b) oxidized MWCNTs and (c) \(\beta\)-cyclodextrin-modified MWCNTs
Table 6.1 Hypochromism constants for pristine and modified MWCNTs calculated from fluorescence intensities

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stern-Volmer Constant, ((K_v) \text{ (M}^{-1}\text{)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>pristine MWCNTs</td>
<td>1036</td>
</tr>
<tr>
<td>oxidized MWCNTs</td>
<td>71</td>
</tr>
<tr>
<td>β-cyclodextrin-modified MWCNTs</td>
<td>1641</td>
</tr>
</tbody>
</table>
6.4 Conclusions

The water-dispersible hybrid material denoted as β-cyclodextrin-modified MWCNTs was achieved by simple conventional covalent bond formation technique. The successful attachment of β-cyclodextrin on the surface of MWCNTs was effectively characterized by conventional techniques like FTIR, TGA, EDS and TEM analyses. This synthetic method provided enough number of β-cyclodextrin units on the surface of MWCNTs. On one hand the procedure affords easy dispersion of CNTs in water, and on the other it serves as nanocavities on the surface of the MWCNT for interaction with the CNT. Then the nanocavities can include either metal ions or organic molecules, which are sufficiently small to ‘fit’ into the β-cyclodextrin cavity, as exemplified by the inclusion of rhodamine 6G and support the potential for using this material for targeting specific sensors.
References


40. C. Y. Yen, Y. F. Lin, S. H. Liao, C. C. Weng, C. C. Huang, Y. H. Hsiao, C. C. M. 
Ma, M. C. Chang, H. Shao, M. C. Tsai, C. K. Hsieh, C. H. Tsai and F. B. Weng, 
Nanotechnology, 2008, 19, 37.

127.

