CHAPTER 3
EXPERIMENTAL PROCEDURES

The main purpose of this study is to form calcium phosphates thin film on a titanium substrate. Some of calcium phosphate compounds which are monocalcium phosphate monohydrate (MCPM), dicalcium phosphate dihydrate (DCPD, Brushite) were used as starting materials. Calcium phosphates thin film was formed by electrochemical deposition based on both aqueous and non-aqueous solutions.

3.1 Raw materials

3.1.1 Electrolyte preparations

The starting materials for electrolyte preparations were monocalcium phosphate monohydrate (MCPM; Fluka with purity ≥ 85%) and dicalcium phosphate dihydrate (DCPD; Fluka with purity ≥ 98%). These materials were excess dissolved in water and 1 molar of phosphoric acid (H_3PO_4), respectively until reach the saturated point. The MCPM saturated in aqueous solution and DCPD saturated in 1M-H_3PO_4 solution would be used as electrolytes for electrochemical deposition in order to form calcium phosphates thin films. The electrolytes were distinguished into 4 main types as described below

1. MCPM saturated in aqueous solution without ions addition.
2. MCPM saturated in aqueous solution with ions addition. These ions were fluoride ion (from NaF; Ajax chemical with purity ≥ 98%) and nitrate ion (from $NaNO_3$; Ajax chemical with purity ≥99%), which were already described in section 2.1.5. The amounts of ions addition into solution were distinguished into three formulas as the followings:

2.1 NaF	0.15 g	$NaNO_3$	21 g → formula I
2.2 NaF	0.10 g	$NaNO_3$	14 g → formula II
2.3 NaF	0.05 g	$NaNO_3$	7 g → formula III

2.3 NaF 0.05 g $NaNO_3$ 7 g → formula III

3. MCPM saturated in 20% and 50% V/V ethanol solutions respectively.
4. DCPD saturated in 1M-H_3PO_4

All the above solutions must be filtered before being used as electrolytes for electrochemical deposition. Theirs pH parameter must be evaluated by pH meter (HI8417 microprocessor Bench pH meter, Hanna instruments). Besides, the titanium plates were cut into rectangular shape of size 2-cm. × 0.8-cm. for use as substrates for film depositions. In addition, these all procedures were described briefly as showed in Figure 3.1.

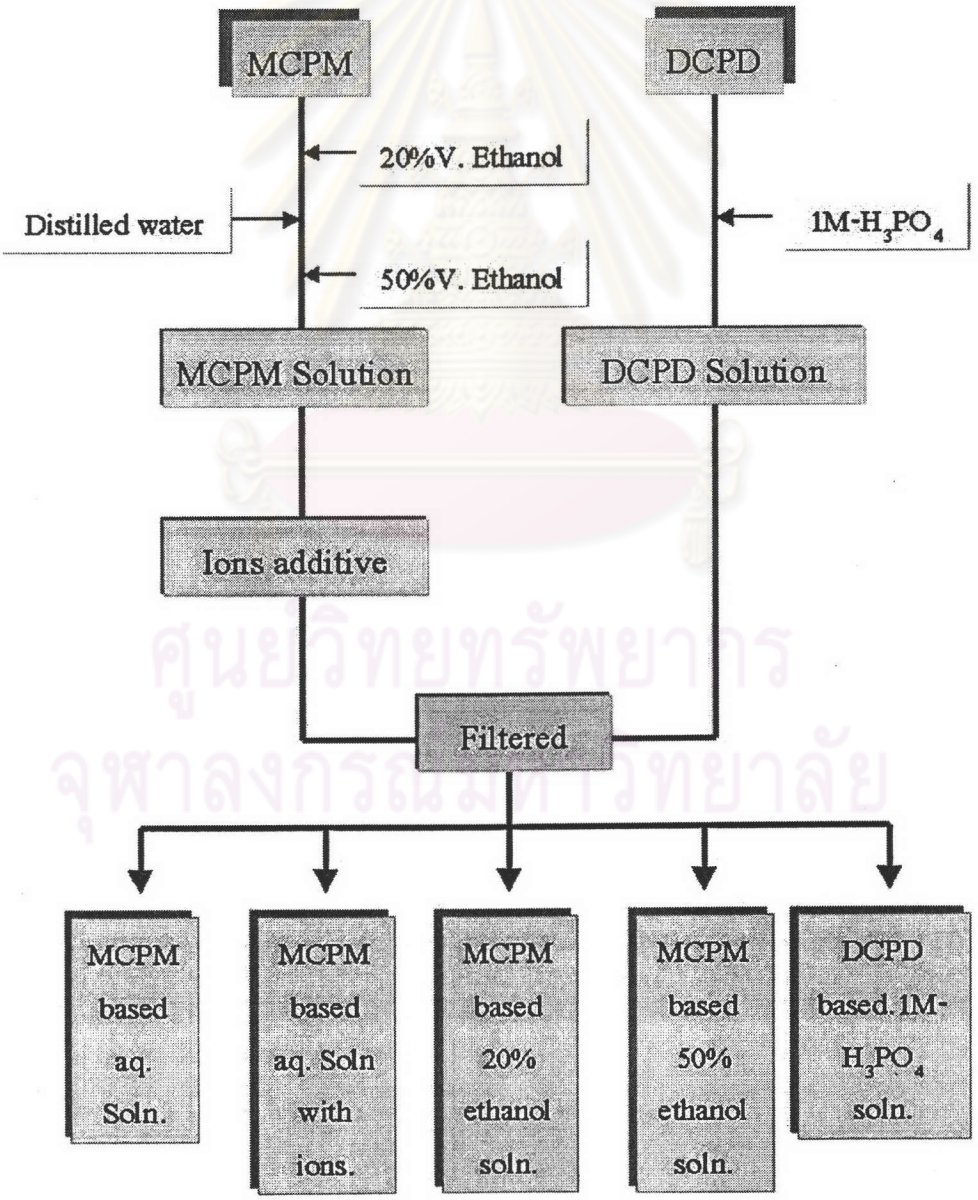


Figure 3.1 Electrolyte preparation flow chart.

3.1.2 Working electrode preparations

The working electrodes were made from pure titanium plates that were cut into rectangular shape with 0.8 × 2-cm. size in order to used as substrates for film deposition. These titanium substrates were connected with copper wires and supported by glass tubes and special araldite glue as shown in Figure 3.2

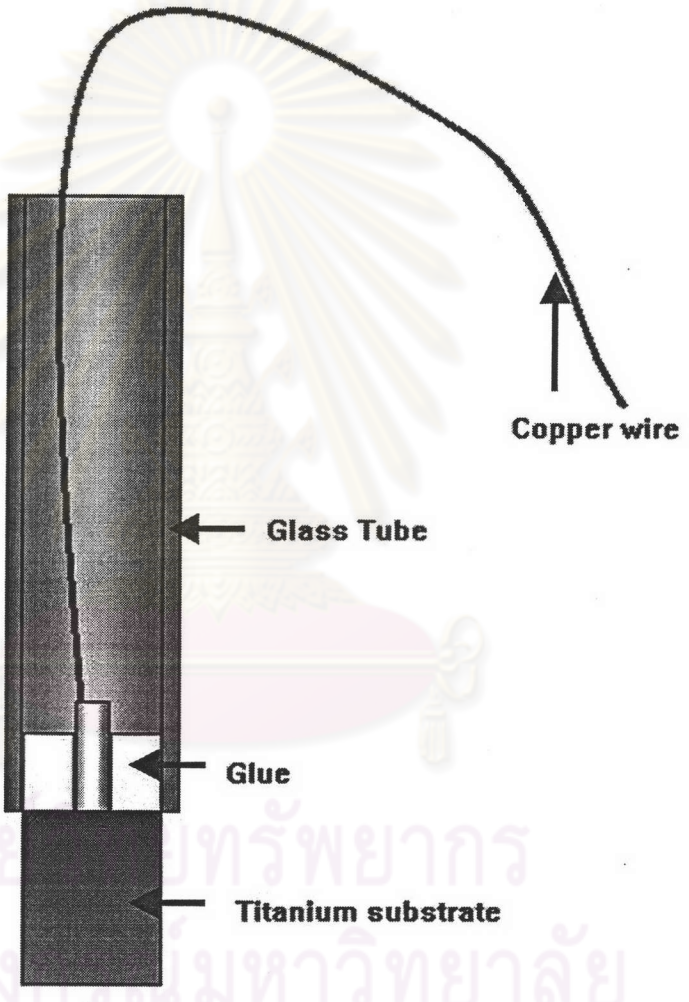
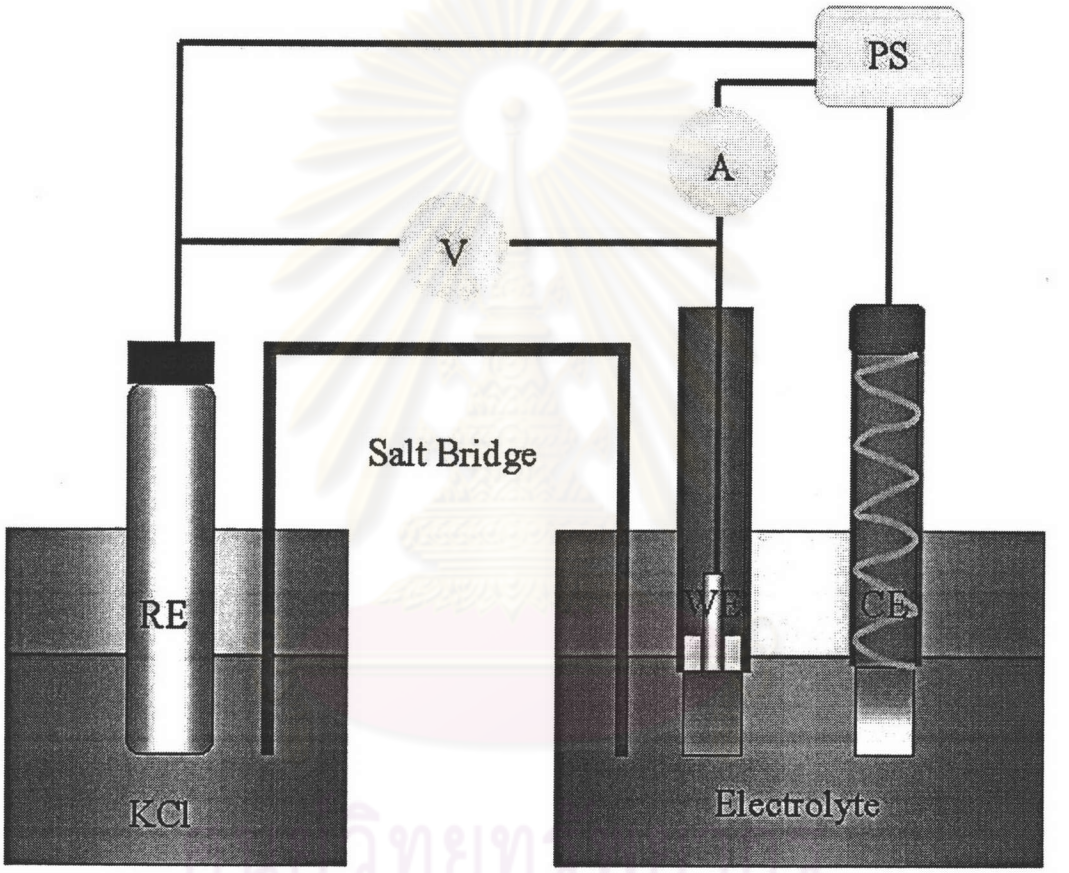


Figure 3.2 The designed working electrode

The titanium substrates must be cleaned by chemical etching with 1 molar of HF for 1 minute, after that, rinsed the substrate well with distilled water before being used as working electrode in every condition of electrolytic depositions.

3.2 Calcium phosphate thin film forming method

The electrolytic deposition was used to form the calcium phosphates thin films. The procedure used in this experiment was set on the Potentiostat model PG-30, Metrohm, with Ag/AgCl reference electrode and Pt-counter electrode. The electrolytic cells were set as showed in figure 3.3



RE: Reference electrode Ag/AgCl

WE: Working electrode Ti

CE: Counter electrode Pt

PS: Potentiostat

Figure 3.3 The electrolytic cells setting in three electrodes system.

Since the electrolyte has been set according to section 3.1, its current density, which was applied by the potentiostat, was varied in a negative region in order to find an optimum current density that could form calcium phosphates film on titanium substrate within 3 and 5 minutes, respectively. Figure 3.4 showed the experimental procedures in this section concisely.

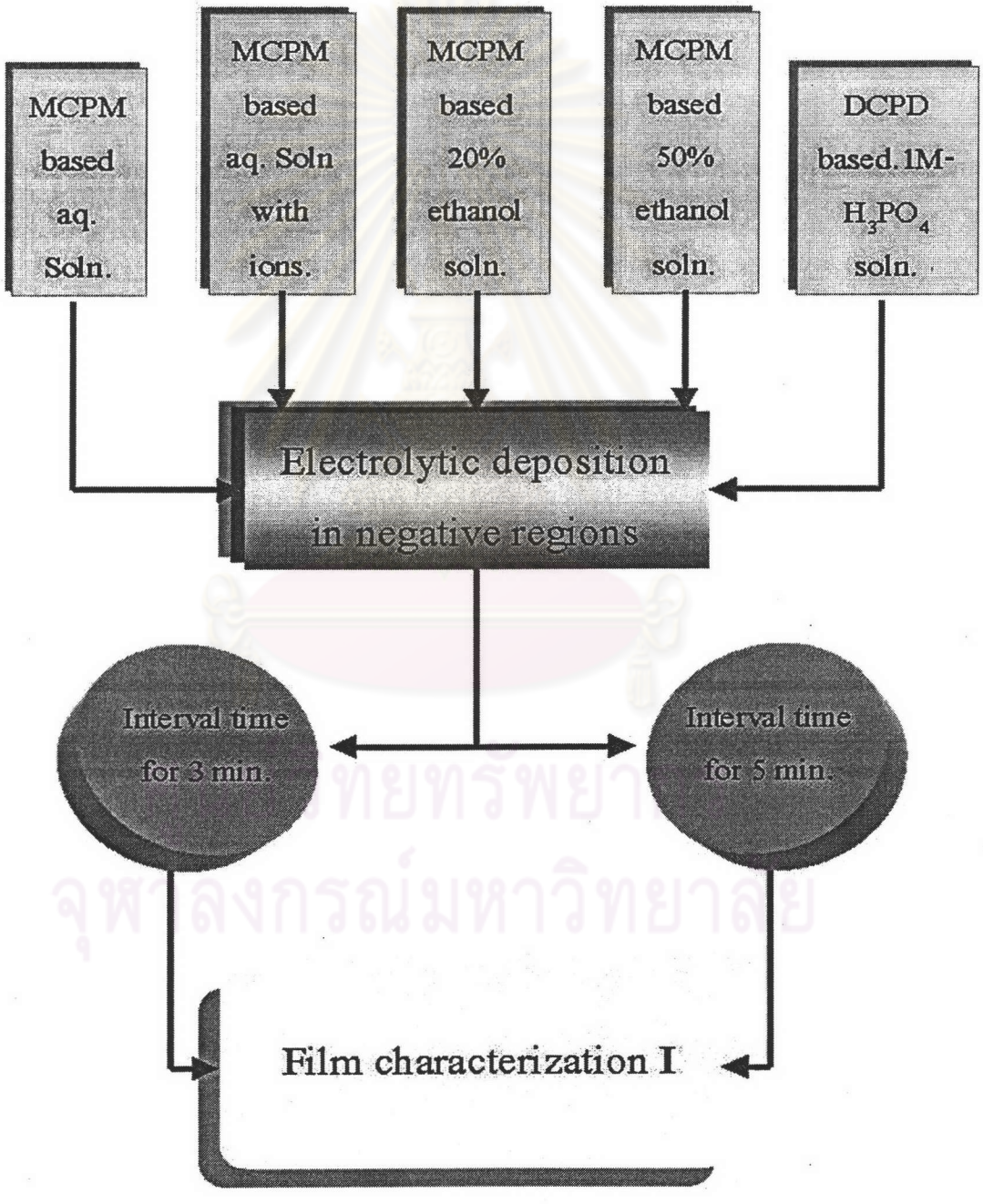


Figure 3.4 Electrolytic depositions flow chart.

3.3 *In vitro* study of biomimetic process.

The specimens from section 3.2 were soaked into R-SBF (according to Kim et.al., 2000) in order to observed calcium phosphates morphology changes with various soaking times. The R-SBF was prepared by adding all substances as showed in Table 3.1 into 1,000 mL of distilled water*.

Table 3.1 Chemicals and their purities and amounts for preparation of 1,000 mL of R-SBF

Reagent	Purity	Amount R-SBF (from Kim et.al.)
NaCl	99.5 %	5.403 g
$NaHCO_3$	99.7 %	0.736 g
Na_2CO_3	99.9 %	2.036 g
KCl	99.5 %	0.225 g
$K_2HPO_4 \bullet 3H_2O$	99.0 %	0.238 g
$MgCl_2 \bullet H_2O$	98.0 %	0.311 g
HEPES**	99.5 %	11.928 g
$CaCl_2$	94.0 %	0.293 g
Na_2SO_4	99.0 %	0.072 g
1M-NaOH	-	1.5 mL

By the way, the soaking times would vary into three deposition times under temperature controlled at 36.5 °C inside of Incubator (Heraeus instrument). Those soaking times were 2 hours, 4 hours, and 8 hours respectively (Figure 3.5).

3.4 Thin film characterization

Calcium phosphate thin films on titanium substrates obtained from section 3.2 and 3.3 were characterized by the following techniques.

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* See Appendix K for more details.

** HEPES = 2-(4-(2-hydroxyethyl-1-piperazinyl)ethane sulfonic acid)

3.4.1 Optical microscope (OM)

The optical microscope (Olympus BX60M Microscope) was used to investigate calcium phosphates as-deposited film surfaces and as-deposited film thickness. In additions, the optical microscope also used to observe the scratched surfaces of specimens after scratch test (section 3.4.4); and also used to investigate the surfaces of specimens after soaking in simulated body fluid.

3.4.2 Scanning electron microscope (SEM)

Scanning electron microscope, SEM (JEOL JSM-5410LV) was used to investigated the microstructure of calcium phosphates thin films. The specimen was stuck on its stub by carbon tape and coated on the surface with gold. Only microstructures of films, both as-deposited and after soaking in SBF, were investigated. The film thickness would be better observed by OM.

3.4.3 X-ray spectrometry (XRD)

X-ray crystallography is one of the most useful methods for exploring the nature of matter. X-ray diffractometry (XRD) was used to determine the phase content in many minerals and materials. Then, in this study, XRD (Bruker, D8-Advance), using CuK_{α} radiation, was used to investigate the obtained phase in calcium phosphates thin film. The XRD data were collected in the range of $2\theta = 5 - 50^{\circ}$, using a step scan of 0.10°.

The specimens, which were formed from every electrolyte in section 3.1.1, must characterized by XRD in comparison with the XRD pattern of the same specimens after soaking in SBF.

3.4.4 Scratch test

The scratch test was conducted to evaluate the coating adhesion. As-deposite specimens were scratch using Balanced Beam Scrape adhesion and Mar tester (SC 8101) which is according to ASTM D-2197. The applied load to loop stylus was starte at 10 g and observed its scratch trace using OM. The shear stress was calculated b the following equation:

$$\tau = \frac{V}{A}$$

When τ = shear stress (Pa)

A = area of the load apply (m^2)

V = applied load (N)

Each specimen would be scratched by metal indenter in every direction (at leas 3 scratch traces per one specimen) at the same load. Then, the maximum load that th coating was not scraped off by the indenter applied, the shear stress could b calculated by the above equation.

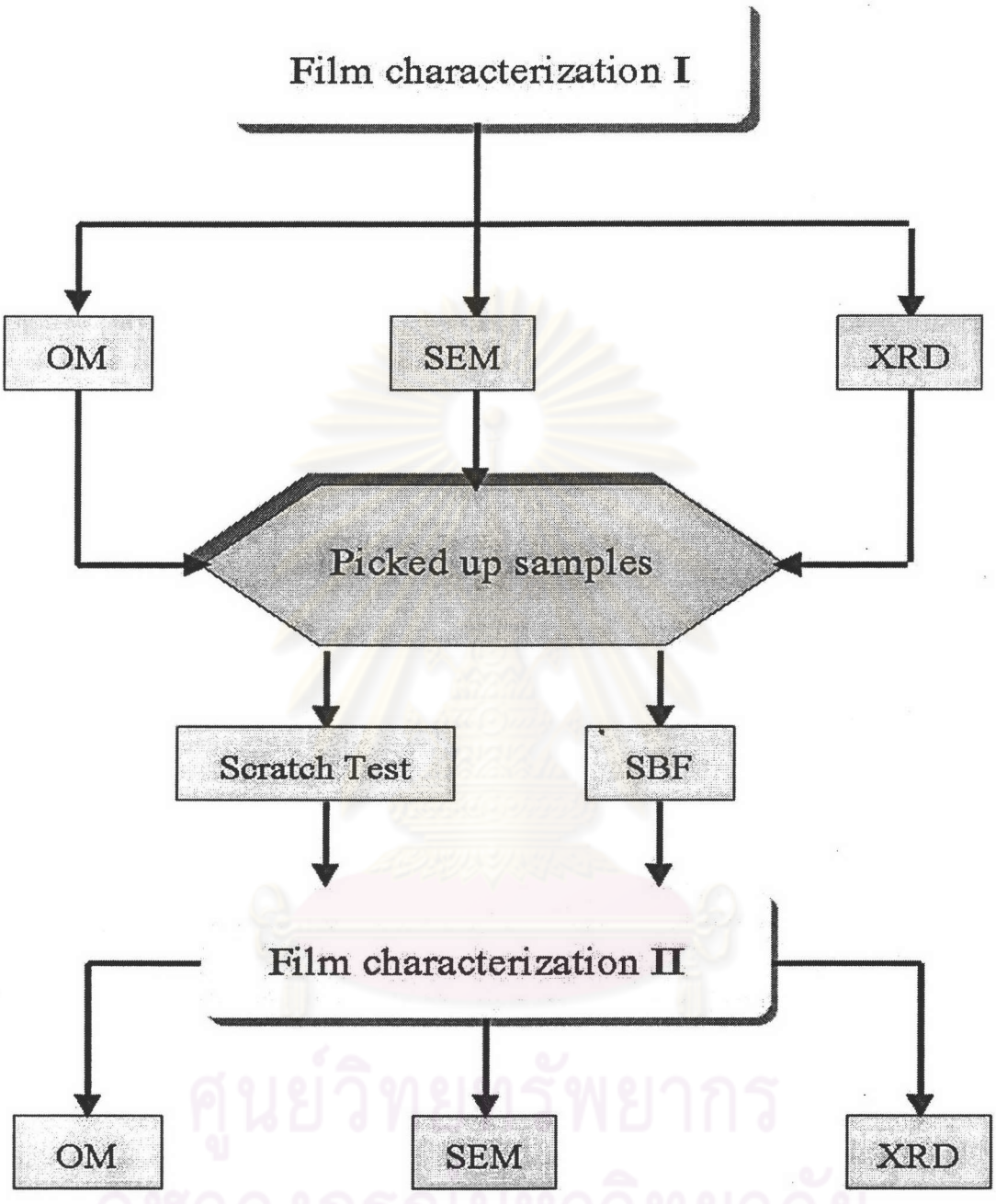

Figure 3.5 Film characterization flow chart.