Aerosol vaccine of Influenza-A Critical Review

Amit B Patil*, Garemella Lakshmi Gayathri

Industrial Pharmacy, Department of Pharmaceutics, JSS College of Pharmacy JSS Academy of Higher education and Research, Mysuru-570015, India

**Article History:**
Received on: 22.Dec.2019  
Revised on: 04.Feb.2020  
Accepted on: 15.Feb.2020

**Keywords:** Influenza virus, Aerosol method, Vaccines, vaccination, localized action, systemic action

**ABSTRACT**
Viruses are the most important mortal pathogens. The influenza virus is accountable for mortality and chronic illness in the world. Today we study the methods of vaccine administration i.e. aerosol method and its evaluation for the improvement of the changes in this method before given to humans and their immunological activity. The aerosol method is taken because it provides the advantages of direct administration of the antigens into bronchi of the lungs thereby producing the antibodies directly for the strains of influenza introduced since its presently nonreplicable viruses. Since vaccination can be done at age of 6 months to 65 years, the aerosol vaccination can be given for the age group of 2-65 years, it can be self-medicated so that dependence is not required as such of the injectables, and it is directed for localized action and there by systemic action. The study also helps in understanding and better the development of vaccines which are useful for the mankind in future, there by chances of eradicating the deadly diseases, this review helps in understanding of the vaccine developments and also in formation of antigen antibodies, comparative studies between the aerosol vaccines, liquid dosage form and solid dosage form help in better understanding of the design of the vaccinations which is useful for all age groups and also useful for the better recovery from diseases and also the effective means for eradication of the viral infections.

*Corresponding Author  
Name: Amit B Patil  
Phone: +91- 9738678943  
Email: amitbpatil@jssuni.edu.in

ISSN: 0975-7538  
DOI: https://doi.org/10.26452/ijrps.v11i2.2184

**INTRODUCTION**
Vaccines is a biological preparation made from an inactive or killed form of virus which causes disease, in order to provide active acquired immunity against same virus.

Influenza is highest infection causing virus in the world which mainly affects the upper and lower respiratory track Figure 1. Influenza is also the most contagious disease which spread as flu, and results in serious respiratory illness that may cause death. Constant changes are seen in the structural form of the virus to attain the resistance against the immunity that body has already developed. (To et al., 2016; Santos et al., 2017).

As a result, various government agencies, public health organizations, including the World Health Organization (WHO), have recommended that yearly influenza vaccination be routinely offered, particularly to people at risk of complications of influenza and those individuals who live with or care for high-risk individuals, including, the elderly (UK recommendation is those aged 65 or above) people with chronic lung diseases (asthma, COPD, etc.) people with chronic heart diseases (congenital heart disease, chronic heart failure, ischaemic heart disease).
people with chronic liver diseases (including cirrhosis)
people with chronic kidney diseases (such as the nephrotic syndrome)
people whose immunity is suppressed (those with HIV or who are receiving drugs to suppress the immune system such as chemotherapy and long-term steroids) and their household contacts
people who live together in large numbers in an environment where influenza can spread rapidly, such as prisons, nursing homes, schools, and dormitories.
healthcare workers (both to prevent sickness and to prevent spread to patients)
pregnant women. However, a 2009 review concluded that there was insufficient evidence to recommend routine use of trivalent influenza vaccine during the first trimester of pregnancy. Influenza vaccination during flu season is part of recommendations for influenza vaccination of pregnant women in the United States (To et al., 2016).

Symptoms for Presence of Viruses
It’s generally self-treatable since the symptoms are common cold, fever, chills, cough and running nose. Patients may also experience Headache, Fatigue, Pain in muscle areas, Dehydration, Loss of appetite, Shortness of breath, Sore throat, Congestion and Swollen Lymph nodes.

The cationic liposomes are produced due to their unique advantages in the process of the immune response. They contain the purified proteins which are favourable for both live and inactive vaccines. By (Table 1) giving the vaccines through the aerosol method the antigens are directly administered into the bronchi of lungs and the production of the antibodies against the given antigens is faster and direct since the respiratory tract mucosal barrier is the strongest route that favours the antibody and cell-mediated immunity development through the layer of mucosal tissues Figure 2, (Santos et al., 2017).

Injection Versus Nasal Spray
Flu vaccines are available either as,

1. a trivalent or quadrivalent intramuscular injection (IIV3 or IIV4, that is, TIV or QIV), which contains the inactivated form of the virus
2. a nasal spray of live attenuated influenza vaccine (LAIV, Q/LAIV), which contains the live but attenuated (weakened) form of the virus.
3. TIV or QIV induce protection after injection (typically intramuscular, though subcutaneous and intradermal routes which can also be protective) based on an immune response to the antigens present on the inactivated virus, while cold-adapted LAIV works by establishing infection in the nasal passages.

Comparative Studies Between Powder and Liquid Dosage
comparison between the powder dosage forms and the liquid dosage.

Reaching higher deposition efficiency is a feature of the powder dose than the liquid dosage.
For powder aerosol there is no need for the additional or extra driving force.
It’s easy for the patients to use than the liquid dosage form.
In previously pre-clinical also the powder inhalation was proven better than liquid aerosol.
The immune response was considered better than the liquid formulations which are delivered intra-muscularly or by pulmonary route.
The flow rate of powders can be monitored than the liquid aerosol formulations.
Liquid feed should be sent through the atomization where the process is exposed to higher temperatures, high stress, and dehydration stress.
The flow rates cannot be monitored for the liquid aerosols, hence the dose rate can be also be expected to be vary.
Hence it is recommended that the use of the powder aerosol than the liquid aerosol.

Nasal route over oral route
Nasal route of administration is considered to be the fastest route of approach compared to another route.
The nasal route is opted since it’s the fastest and dose can be directly reached to the lungs or respiratory system.
Whereas in the oral route of administration the onset action time is more than considered.
Through oral route the chances of side effects for the other body parts are more (Sham et al., 2004; Saluja et al., 2010).

Formulation Liposomes
According to atal. Slütter B, Bal SM, Ding Z, Jiskoot W, Bouwstra JA the formulation is done as follows
By using the fluorocarbon in water the microparticle shell is prepared using the spray drying process. In this method, the emulsion was made,
Figure 1: Structure of Influenza Virus

Figure 2: Starting of Infection Process in Humans
Figure 3: The coding of the DNA of the influenza viruses for the formation of the vaccines.

Table 1: Types of Mucosal Vaccines available in Markets

<table>
<thead>
<tr>
<th>Type of Vaccines</th>
<th>Trade Names for vaccines (Developer)</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza type A and</td>
<td>FluMist (Med Immune)</td>
<td>Intrasanal route</td>
</tr>
<tr>
<td>Influenza type B viruses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1N1 influenza virus (swine flu)</td>
<td>NASOVAC (Serum Institute of India)</td>
<td>Intrasanal route</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>RotaTeq (Merck); Rotarix (Glaxo-Smithkline)</td>
<td>Oral route</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Many</td>
<td>Oral route</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>Vivotif (Crucell); Ty21A</td>
<td>Oral route</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>Orochol (Crucell)</td>
<td>Oral route</td>
</tr>
<tr>
<td>Cholera</td>
<td>Dukoral (Crucell); Shanchol (Shantha Biotechnics)</td>
<td>Oral route</td>
</tr>
</tbody>
</table>
Table 2: Central Composite Design with independent input variables

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Hydration volume</th>
<th>Hydration time</th>
<th>Drug: lecithin Ratio</th>
<th>Sonication time(mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105</td>
<td>30</td>
<td>0.1:3.5</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>45</td>
<td>0.1:2</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>45</td>
<td>0.1:2</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>45</td>
<td>0.1:6</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>105</td>
<td>15</td>
<td>0.1:3.5</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>30</td>
<td>0.1:3.5</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>105</td>
<td>30</td>
<td>0.1:6</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>15</td>
<td>0.1:6</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>120</td>
<td>15</td>
<td>0.1:6</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
<td>45</td>
<td>0.1:6</td>
<td>14</td>
</tr>
<tr>
<td>11</td>
<td>105</td>
<td>30</td>
<td>0.1:2</td>
<td>21</td>
</tr>
<tr>
<td>12</td>
<td>105</td>
<td>30</td>
<td>0.1:3.5</td>
<td>28</td>
</tr>
<tr>
<td>13</td>
<td>120</td>
<td>15</td>
<td>0.1:2</td>
<td>28</td>
</tr>
<tr>
<td>14</td>
<td>105</td>
<td>45</td>
<td>0.1:6</td>
<td>21</td>
</tr>
<tr>
<td>15</td>
<td>105</td>
<td>30</td>
<td>0.1:3.5</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>120</td>
<td>30</td>
<td>0.1:3.5</td>
<td>21</td>
</tr>
<tr>
<td>17</td>
<td>90</td>
<td>15</td>
<td>0.1:2</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 3: Vaccine Formulation by Administration route

<table>
<thead>
<tr>
<th></th>
<th>OVA (µg)</th>
<th>CpG (µg)</th>
<th>Liposomes (µg)</th>
<th>Total volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcutaneous</td>
<td>100</td>
<td>100</td>
<td>5000</td>
<td>70</td>
</tr>
<tr>
<td>Nasal</td>
<td>20</td>
<td>20</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>Intradermal</td>
<td>2.0</td>
<td>2.0</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>Intranodal</td>
<td>0.20</td>
<td>0.20</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

stabilized by using the saturated lipid as material (Lipoid, Germany) (derivatives of phosphatidylcholine derivatives, DPPC and DSPC; EPC3 previously described (Dellamary et al., 2000; Glück et al., 1999).

During spray drying process, solutions of stephydroethyl starch and antigens are added which are used as hydrophilic agents and lactose and calcium chloride which are used as the other nonactive excipients. The particle size was supposed to be in 1 and 5 and density is about less than 0.2g/cm3 Figure 3.

A similar process is used for the formulation of the Fluzone, which contains the protein antigen and tyloxapol present in 0.5% and 1% respectively.

Characterization

Scanning electron microscopy and densitometry techniques are used for the microparticles description. The process used is as follows,

The sterile buffer PBS is taken and ten SDM were suspended which contained the antigen, which is for the description and they are incubated at 37°C under slow shaking conditions. The samples are formed which have the ranges of the time and centrifuged and the supernatants are stored at -70c. The PBS solution containing the 0.1% SDS and the liquid pallets are resuspended in it and the old shaking at room temperature for a time of 30 mins and the recentrifuged samples are removed from the solution and accordingly, the protein concentration was determined using the assay process and using the bioassay method the TcH specific and HA110-120 peptides are used and cultured with the M12 B Lymph cells used for the antigen percent present.

Preparation Liposomes

Atal. Slütter B, Bal SM, Ding Z, Jiskoot W, Bouwstra JA proposed this method where we can get to know the different methods of preparation of liposomes this method is as follows,

The film hydration method is used for the preparation of the liposomes (Bangham et al., 1989) which

is then concluded with extrusion (Bal et al., 2011). By using the PC, DOTAP, and DOPE film was formed by using the rotary evaporator the chloroform which was added is evaporated and thereby washing with nitrogen. Ratios used is (9:1:1 molar ratio).

By using phosphate buffer pH 7.4 (7.7 mM Na2HPO4 and 2.3 mM NaH2PO4) 10 mM, containing OVA1.5 mg/ml the film was rehydrated. After the lipid formation 5%(w/v), the rehydration process takes place by adding CpG (final concentration of 1.5 mg/ml) and freeze-dried the dispersions were followed by 10 mM phosphate buffer pH 7.4 used in rehydration. extrusion was done for 4 times through two different pore size filters i.e. 400nm and 200nm for the formation of the liposomes in equal size in solution.

**Characterization**

Using a Zetasizer® Nano ZS (Malvern Instruments, UK), the size of the liposomes can be determined by dynamic light scattering (DLS) and the zeta potential can be measured by laser Doppler velocimetry (Amidi et al., 2006).

**Evaluation of novel aerosol formulations**

**Evaluation test for liposomes**

**Size reduction**

It is evaluated by Sonicator, generally probe sonicator is used for the estimation. Based on the delivery of the powder amplitude, it is set for about 40%, at room temperature and time intervals 14,21,28 mins (Table 2)

Particle size: Its measured by the zeta sizer nano instrument

**Determination of Zeta Potential**

Instrument used is dynamic light scattering used for the zeta potential estimation (Malvern Instruments Inc, Malvern, UK). It is estimated in room temperature and refractive index used is 1.33. the liposomal solution is added in tubes without air bubbles for their measurement.

**Determination of entrapment efficiency**

The entrapment of the drug in the liposomes is estimated by using centrifugation technique, the pellets are centrifuged for 4000 rpm for 18 mins there by the drug is released into centrifugation tube and the process is repeated again at 2000 rpm for 38 mins and tempartature is to be 4°C. The drug is mixed with methanol and water and it is estimated by HPLC instrument (Table 2).

Percentage Entrapment Efficiency

\[
\text{Percentage Entrapment Efficiency} = \left( \frac{W_c}{W_t} \right) \times 100
\]

**Determination of Drug loading**

The liposomal solution is dissolved in the methanol and water solution there by the solution is used for determination of the drug present in them at various levels its estimated by HPLC instrumentation pipes used are 0.22 micrometers.

**Vaccination**

8 week old female BALB/c mice (Charles River, Maastricht, The Netherlands) received OVA or OVA-and CpG-containing formulations via transcutaneous, nasal, intradermal or intranodal administration (Table 3).

**Animal Studies**

The animal studies are performed to evaluate the activity of the formulation prepared and tested and to know if any further changes are required, which when done, they can be comfortably used for human administration.

According to these studies, the female rate species {BALB} and Sprague Dawley rats are taken for testing from (Indianapolis, IN) Harlan Sprague Dawley, were conserved in the precise microorganism-free, Alliance Pharmaceutical Corp which is qualified of AAALAC. "Principles of Laboratory Animal Care" and are observed in the experiments studied they’re and were approved by the Institutional Animal Care and Use Committee (Dellamary et al., 2000).

**Advantages of using mice for animal studies**

1. They are low cost
2. They are easily available
3. Small in size and easy to handle
4. They can be reared easily
5. They are well suited for the animal studies
6. There is the availability of the transgenic mice and can be easily mutated
7. Well suited for alternative routes of administration.

**Antigens**

This testing method is important for the vaccines which are to be tested because they are the ones which produce the antibodies for the given antigen strain. The method is as follows,

In this testing method on tolerant (MDBK), the WSN strain of influenza virus are grown on the Madin–Darby bovine kidney carcinoma cells. The A/WSN/32 H1N1 viruses was recuperated and...
supernatant by sucrose-gradient centrifugation in sterile phosphate buffered saline (PBS), they are resuspended and ultraviolet (UV) short-wave light used for the inactivation which is done twice standard titration on tolerant Madin–Darby canine kidney (MDCK) carcinoma cells can confirm the Inactivation. The amount of viral antigen was measured in a bioassay using M12 B lymphoma cells as antigen-presenting cell and a T cell hybridoma specific for HA 110–120 peptide (Bot et al., 1996) a sterile preparation is made for intramuscular use from the influenza viruses for the influenza vaccines which are administered into the chick embryo, (strains: A/New Caledonia/20/99(H1N1), A/Panama/2007/99(H3N2) and B/Victoria/504/2000(ZonalPurified,Subvirion) Fluzone® (AventisPasteur,Swiftwater), PA for the process of inactivation formaldehyde is used on the virus-containing fluids. By the process of the linear sucrose density gradient solution, the influenza viruses are purified after extraction. By using polyethylene glycol Piso-octyl phenyl the virus was chemically disrupted producing a "split-antigen".

In isotonic sodium chloride solution and sodium phosphate-buffered the split-antigen was added and purified by chemical means, by the manufacturer, at a concentration of 45_g hemagglutinin (HA)/0.5 ml dose of Fluzone® was suspended in saline solution.

This method of testing the viruses antigen testing for influenza virus is taken from the review of intranasal vaccination.

According to this method, (H1N1: A/Brisbane/59/07; A/New Caledonia/20/99 and H3N2: A/Brisbane/10/07) and MNA and with NIBSC reference HAI assays were performed delNS1-H5N1 (A/Vietnam/1203/04-like) vaccine candidate viruses (Romanova et al., 2009).

Immunization

According to this method, the immunization can be calculated as follows: this method is taken from the novel aerosol formulation and design. In rats for intra-tracheal insufflation by dry powders, delivered directly into trachea insufflator which contains the 1 mg of powder and given into lungs using 2ml of air, so it reached the pulmonary tree directly. (Penna-Century Inc., Philadelphia, PA).

The antigens are suspended in the sterile saline and given in the lungs using the same pattern. In mice, the parenteral administration carried out subcutaneously, at specified time intervals from the veins of the rats the blood is taken from tail veins with bronchoalveolar fluid harvested by large with heparinized phosphate buffer saline up on animal sacri-

face. There are other processes for the immunological studies which are described in another review point of where they are performed as follows.

ELISA (Enzyme-linked immunosorbent assay)

This method is taken from Intranasal vaccination neutralizing mucosal IgA antibodies in humans which induce hetero-subtypic.

Total mucosal IgA and IgG ELISA, specific mucosal IgA and serum IgG as well as using microtiter plates are coated with, anti-human IgA or IgG (Sigma) and IgA detection antibodies (Sigma) or HRP-conjugated anti-human IgG, recombinant hemagglutinin (Sino Biological), were performed as mentioned above (Bot et al., 1996).

Precise ELISA test results were stated in random units (AU/ml)depending on the reference standard with that of the calibration curve (pooled serum or nasal wash) by the 4 parameters logistic fit results were normalized to total IgA of Specific mucosal IgA 1_g (Gen5 v2.1, BioTek) (Morokutti et al., 2014).

HAI (Hemagglutination inhibition assay)

A standard protocol from WHO which it analyzed was applied, 0.8% of human erythrocytes and adding 4 HAU/25_l of virus antigen to serially diluted serum for 2 times (Romanova et al., 2009).

The last serum dilution of the HAI titer is the reciprocal that inhibits hemagglutination. At a 60◦ angle plate were analyzed and (Morokutti et al., 2014).

MNA (Microneutralisation assay)

The WHO has set a protocol for the MNA which was adapted (Romanova et al., 2009) for determination of this method.

The samples were serially diluted for 2 times, then virus antigen 100 TCID50/5_l was added and incubated for 120 ± 30 min.

The assay of the cells s carried out (2 × 104cells/well) i.e.Vero or MDCK were then added and the sample is incubated overnight (20 ± 2 h) before they use acetone for stabilizing. The detection of anti-influenza for A viral infection combination with (Chemicon) in polyclonal goat anti-mouse IgG conjugated withHRP (KPL), nucleoprotein-specific monoclonal mouse antibody and stained with TMB (KPL). Stopped plates (4 M H2SO4)are photo metrically measured (BioTek) at 450 nm and for the reciprocal of the dilution that neutralizes 50% of viruses antigen, the MNA titers are used (Morokutti et al., 2014).

Determination of Serum Secretory IgG1, IgG2a, IgA IgG, and IgG

This method is used for determination of the
immunological studies which are conducted for the
determination of the immunoglobulins.

The coated Microtiter plates (Nunc, Roskilde, Den-
mark) placed overnight 4°C pH of 9.4 100Mm car-
bonate buffer with 100ng of OVA per cell.

Wells was to be blocked with 1% BSA in phosphate
buffer solution for a time period 1 h at a temperature
of 37 °C.

Thereby OVA-specific antibodies were detected
using HRP conjugated goat anti-mouse IgG, IgG1,
IgG2a or IgA from the serial dilutions that were
taken place for 1.5 h after. The determination
of Enzyme activity is done by incubating with
TMB/H2O2.

In acetate buffer pH 5.5 at 100 mM for 15 min at
room temperature. The reaction is stopped with
H2SO42 M and at 450 nm with an EL808 microplate
reader absorbance can be checked (Bio-Tek Instru-
maments, Bad Friedrichshall, Germany) (Slütter
et al., 2011).

**Antigen-Specific DCs in Lymph Node**

This is used for the determination of specified anti-
gen in the lymph node which is useful in the treat-
ment of the patients and the method is as follows,

The vaccination given for the mice with the prepara-
tion mentioned as per (Table 3), but OVA was substi-
tuted with OVAFF647 and CpG with CpGFITC.

The mice are sacrificed for the testing and their
lymph nodes are taken, This is done after 4 or 24 hrs
of time from vaccination given (for nasal the cervical
lymph nodes intradermal and intranodal adminis-
tration the inguinal and for transcutaneous) and the
susension having single cells are obtained. Cells
found were washed with FACS buffer and stained
with anti-CD11c-PE-Cy7 to allow detection of DCs.

Using flow cytometry (FACSCantoll, Becton Dickin-
son), the amount of OVAFF647+ and CpGFITC + DCs
was determined (Slütter et al., 2011).

**Measurement of T cell response**

T cells have greatest significance, the cell mediat-
ing immunity hence their action on administration is
an important aspect; hence the standard test is per-
formed, which is as follows,

On day 40 after giving a vaccination, the rats which
are immunized are taken and their lymph nodes are
hygienically separated and used for testing. The
cells are isolated and they are stored in the solu-
tion of 5 x 106 ml-1for time period of 3 days with
antigens or cell culture solution. The amount of
the cytokines are measured by (Biosource Interna-
tional, Camarillo, CA).

**ELISA test method**

In various experimental processes, from immu-
nized mice, pulmonary interstitial APC was isolated
by method collagenase digestion, (Sigma) gradi-
ent centrifugation and positive selection with (Mil-
tenyi Biotech, Germany) anti-MHC class II magnetic
beads.

The Flow cytometric analysis was carried out using
PE-tagged anti-CD11c, anti-CD11b and the instru-
ment used is a FACS Caliber.

The quantitation of activated TcH was carried and
Incubation of HA-specific T cell hybridoma with APC
cells and out as described previously (BD PharMin-
gen, San Diego, CA) (Santos et al., 2017).

In some cases, M12 B lymphoma cells (H-2d) pulsed
ex vivo with antigens or microparticle extracts
obtained are SDS-free, were used as APC (Smith
et al., 2003).

**CONCLUSIONS**

Form the above-discussed review methods, the
aerosol method of giving vaccination is preferable
and they help in the fast development of the antibodi-
es against the given antigen strain of the influenza
viruses and it gives the most effective method of the
drug delivery into the respiratory tract and thereby
having an excellent bio-availability in the lymphoid
tissues. It also helps in the fast recovery of the prob-
lems caused.

**REFERENCES**

Amidi, M., Romeijn, S. G., Borchard, G., Junginger,
H. E., Hennink, W. E., Jiskoot, W. 2006. Prepa-
ration and characterization of protein-loaded N-
trimethyl chitosan nanoparticles as nasal deliv-
ery system. *Journal of Controlled Release*, 111(1-

Bal, S. M., Hortensius, S., Ding, Z., Jiskoot, W.,
Bouwstra, J. A. 2011. Co-encapsulation of anti-
gen and Toll-like receptor ligand in cationic lipo-
somes affects the quality of the immune response
in mice after intradermal vaccination. *J Immunol*

Bot, A., Bot, Antohi, K. K., B, C. 1996. Kinetics of
generation and persistence on membrane class II
molecules of a viral peptide expressed on foreign

2254 © International Journal of Research in Pharmaceutical Sciences


